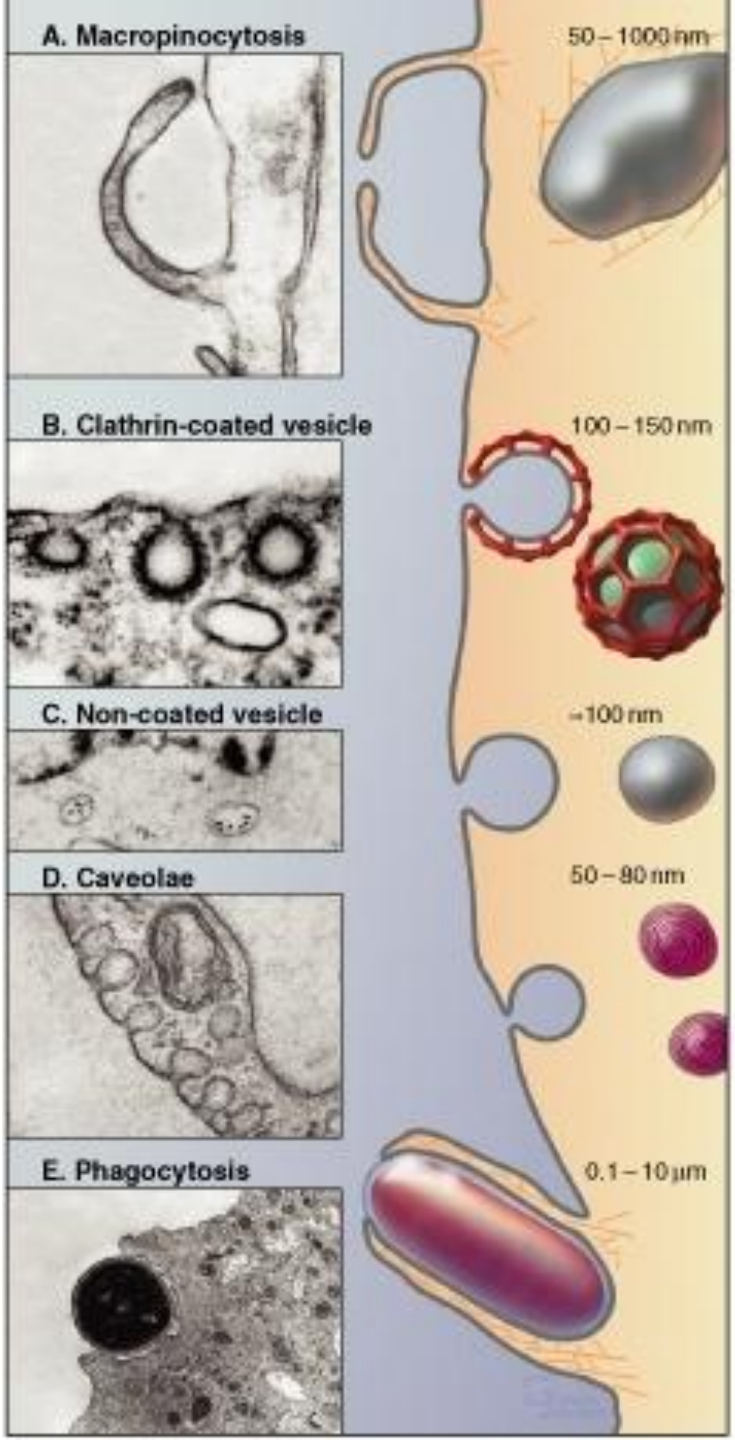
VESICULAR TRANSPORT AND SORTING OF MOLECULES

Some of the functions of endocytosis

- Nutrient uptake
- Receptor recycling
- Plasma membrane protein downregulation and/or degradation
- Synaptic vesicle recycling
- Transcellular signaling
- Exploitation: virus and toxin entry into cells

Common features of endocytosis

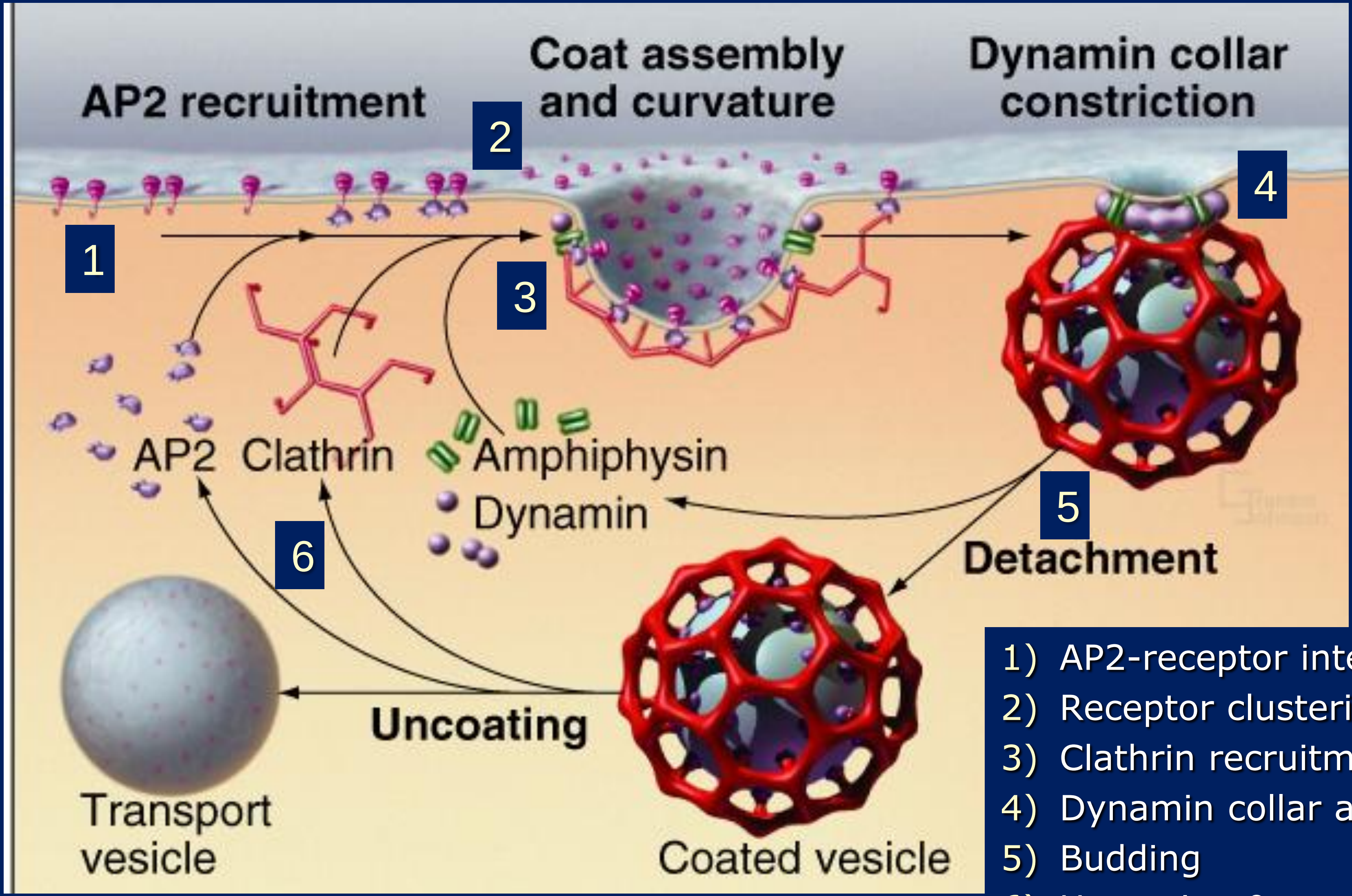
- Small region of the plasma membrane invaginates to form *membrane-limited vesicles* (0.05-0.1 mm diameter)
- Internalized molecules *retain topology* (lumenal = extracellular)
- Cargo can be *specifically selected*
- Destination of cargo can be *controlled*; destination depends on pathway of uptake
- Cargo *receptors can be recycled* to the cell surface
- Conditions under which internalization occurs can be regulated
- Cytoskeletal and coating proteins involvement



Endocytic Mechanisms

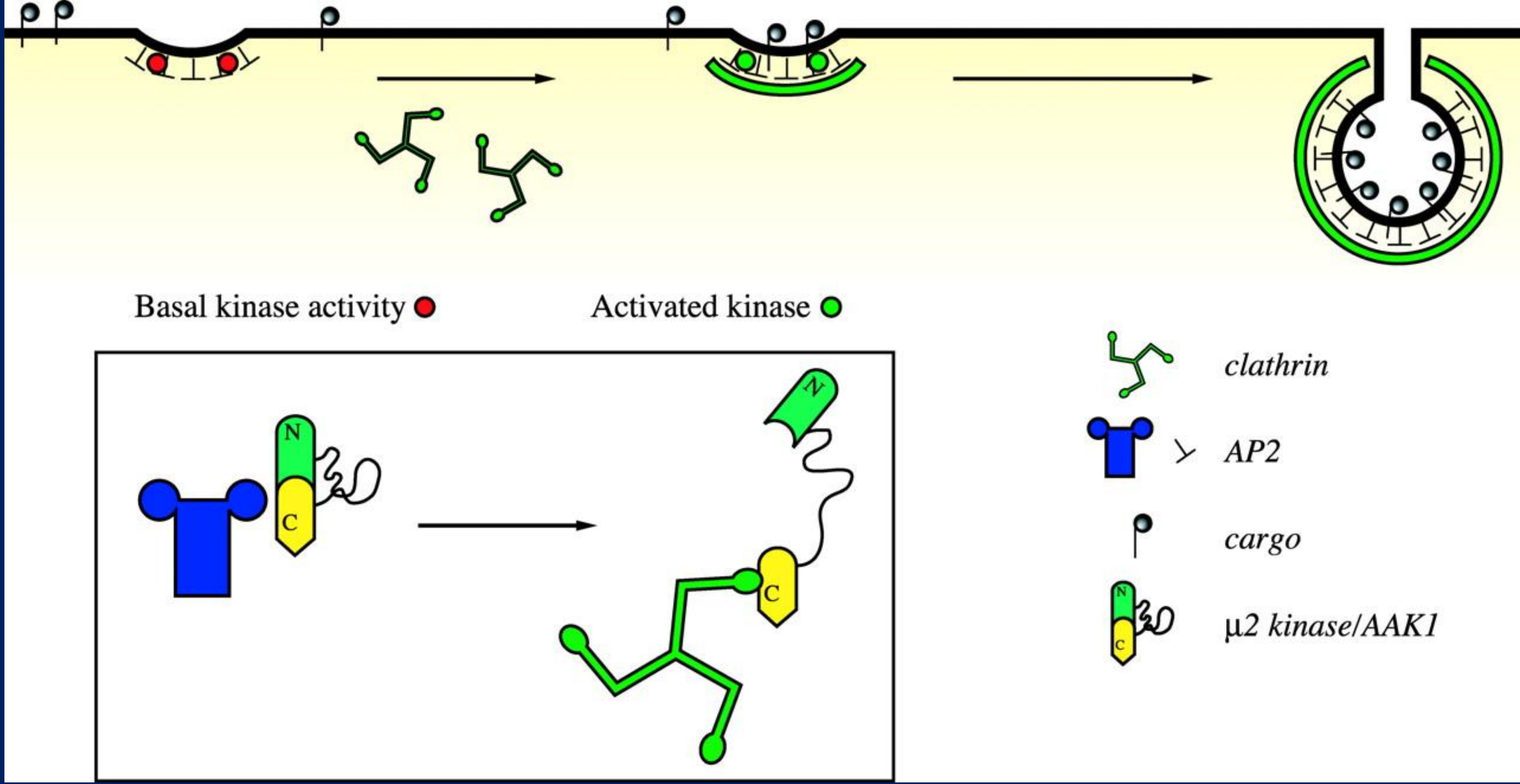
- clathrin coated pits
- caveolin coated caveolae
- non-coated pit pinocytosis
- cytoskeletal derived
  - macropinocytosis
  - phagocytosis

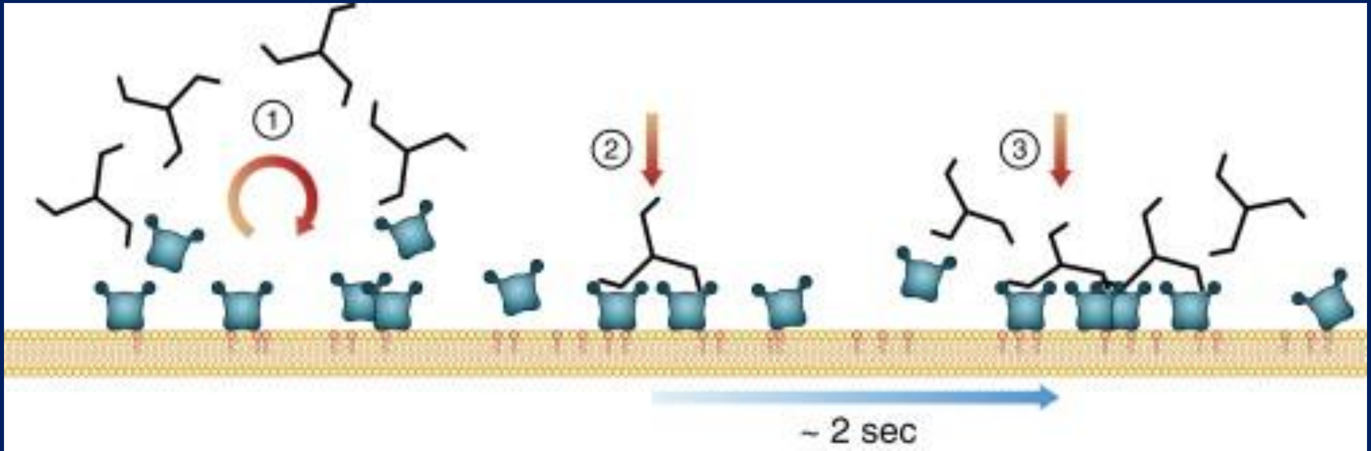
Formation of Coated Pit

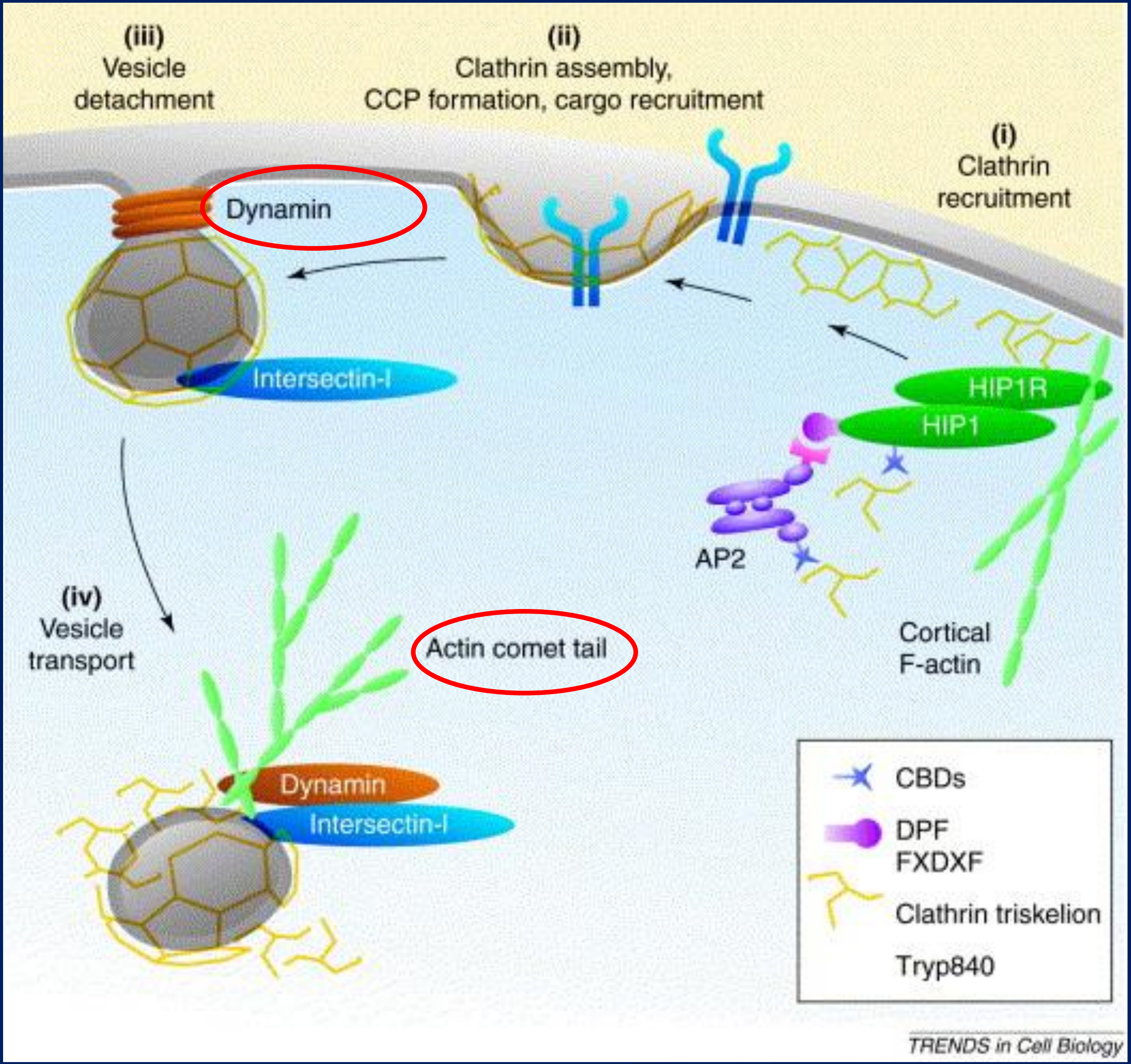


1) AP2-receptor interaction
2) Receptor clustering
3) Clathrin recruitment
4) Dynamin collar assembly
5) Budding
6) Uncoating & recycling of components

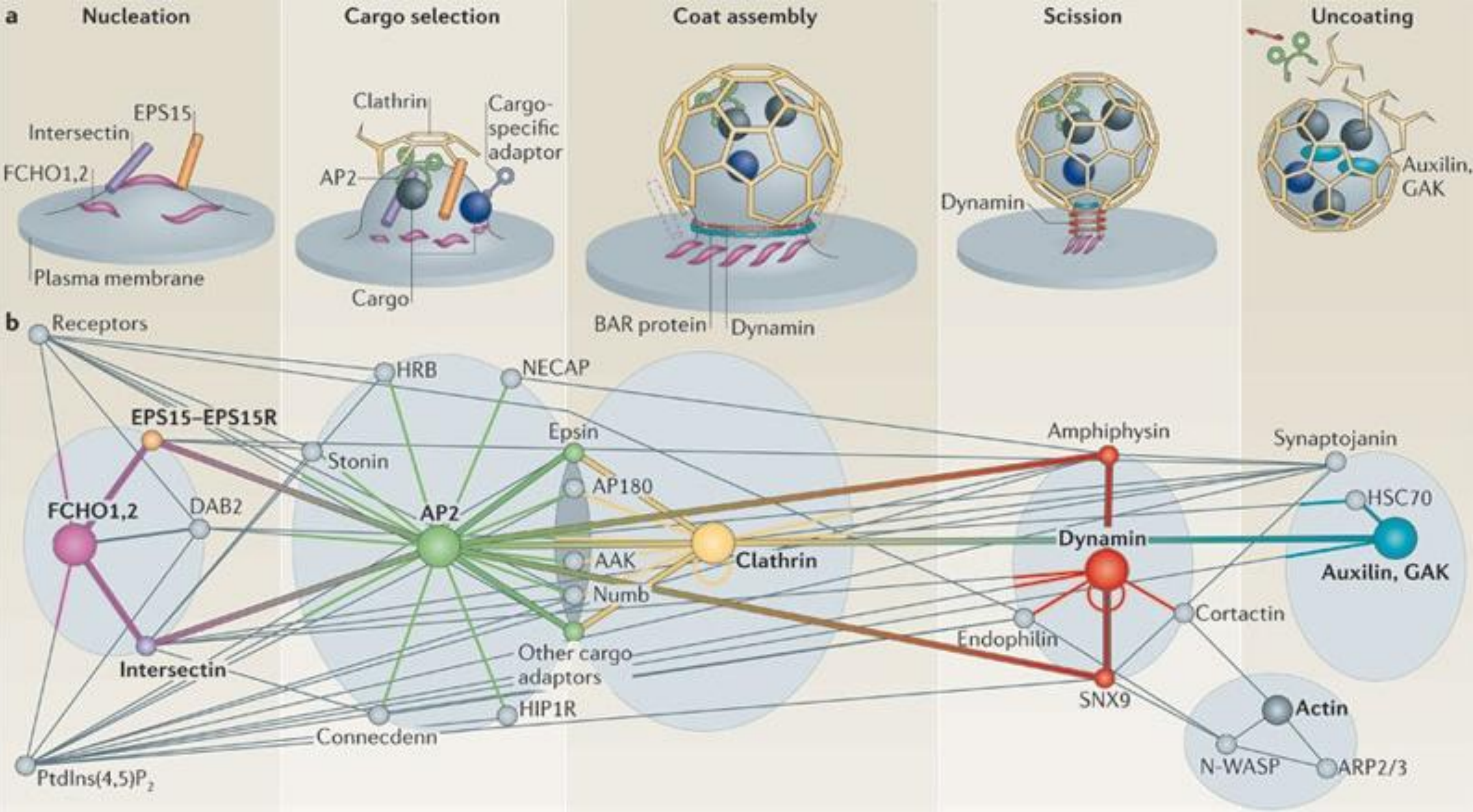
AP=accessory protein


Basal kinase activity ●
Activated kinase ●
N
C
N
C
clathrin
AP2
cargo
µ2 kinase/AAK1

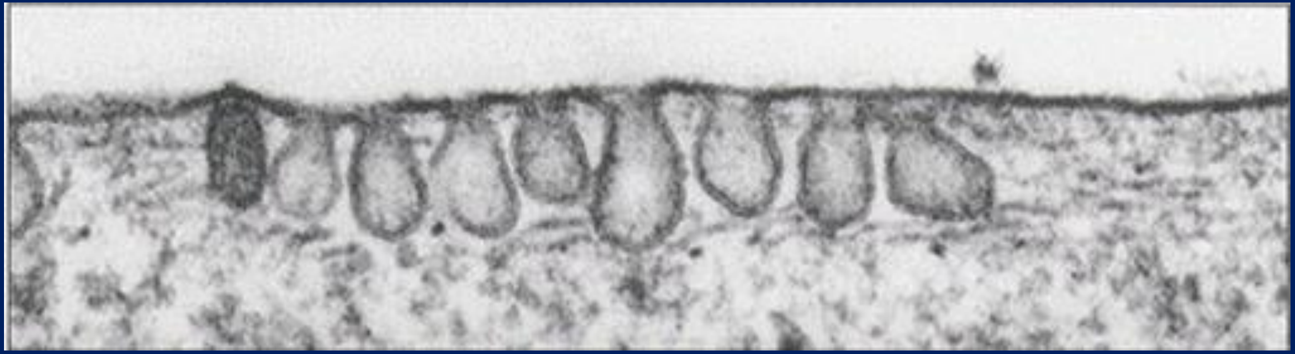

①
②
③
~ 2 sec



TRENDS in Cell Biology


a
Nucleation
Cargo selection
Coat assembly
Scission
Uncoating
EPS15
Intersectin
FCHO1,2
Plasma membrane
Clathrin
Cargo-specific adaptor
AP2
Cargo
BAR protein
Dynamin
Dynamin
Auxilin, GAK
b
Receptors
HRB
NECAP
EPS15–EPS15R
Stonin
Epsin
Amphiphysin
Synaptojanin
AP180
HSC70
FCHO1,2
DAB2
AP2
Clathrin
Dynamin
Auxilin, GAK
AAK
Numb
Cortactin
Endophilin
Intersectin
Other cargo adaptors
SNX9
Actin
HIP1R
Connecdenn
N-WASP
ARP2/3
PtdIns(4,5)P2

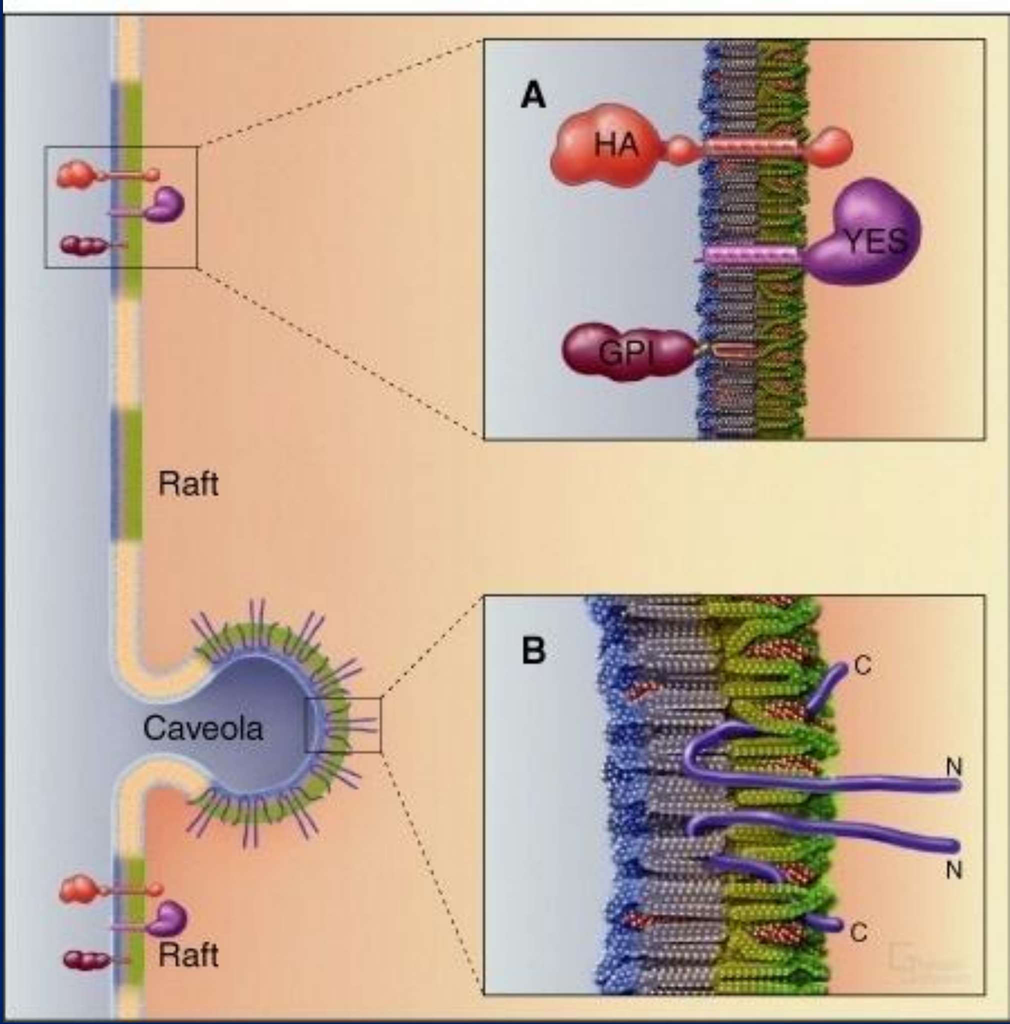
Caveolae



- Caveolae are 50-100 nm invaginations on the cells surface
  - Caveolin, a membrane protein, is the "coat protein" of caveolae
- Do not undergo constitutive endocytosis but can undergo ***endocytosis in response to a signal*** (ex. SV40 binding) in a cholesterol- and dynamin-dependent fashion
- Internalized caveolae recruit actin to form "comet tails"
- Upon internalization caveolae are delivered to novel endosomal compartments known as "caveosomes"

Caveolae

- Associated with lipid rafts
- Membrane lipid cycling
- **Raft-associated protein endocytosis/exocytosis**
  - Inner leaflet proteins
    - acylated proteins
    - prenylated proteins
  - Outer leaflet proteins
    - GPI-linked
  - Some TM proteins that interact specifically with glycosphingolipids/cholesterol
- Caveolin
  - **high-affinity cholesterol binding protein**



Clathrin viz caveolae endocytosis

- ❑ Both caveolae and clathrin-mediated endocytosis involves dynamin
- ❑ Caveolae endocytosis can be inhibited by dominant negative caveolin
- ❑ Caveolae endocytosis is inhibited by cholesterol depletion

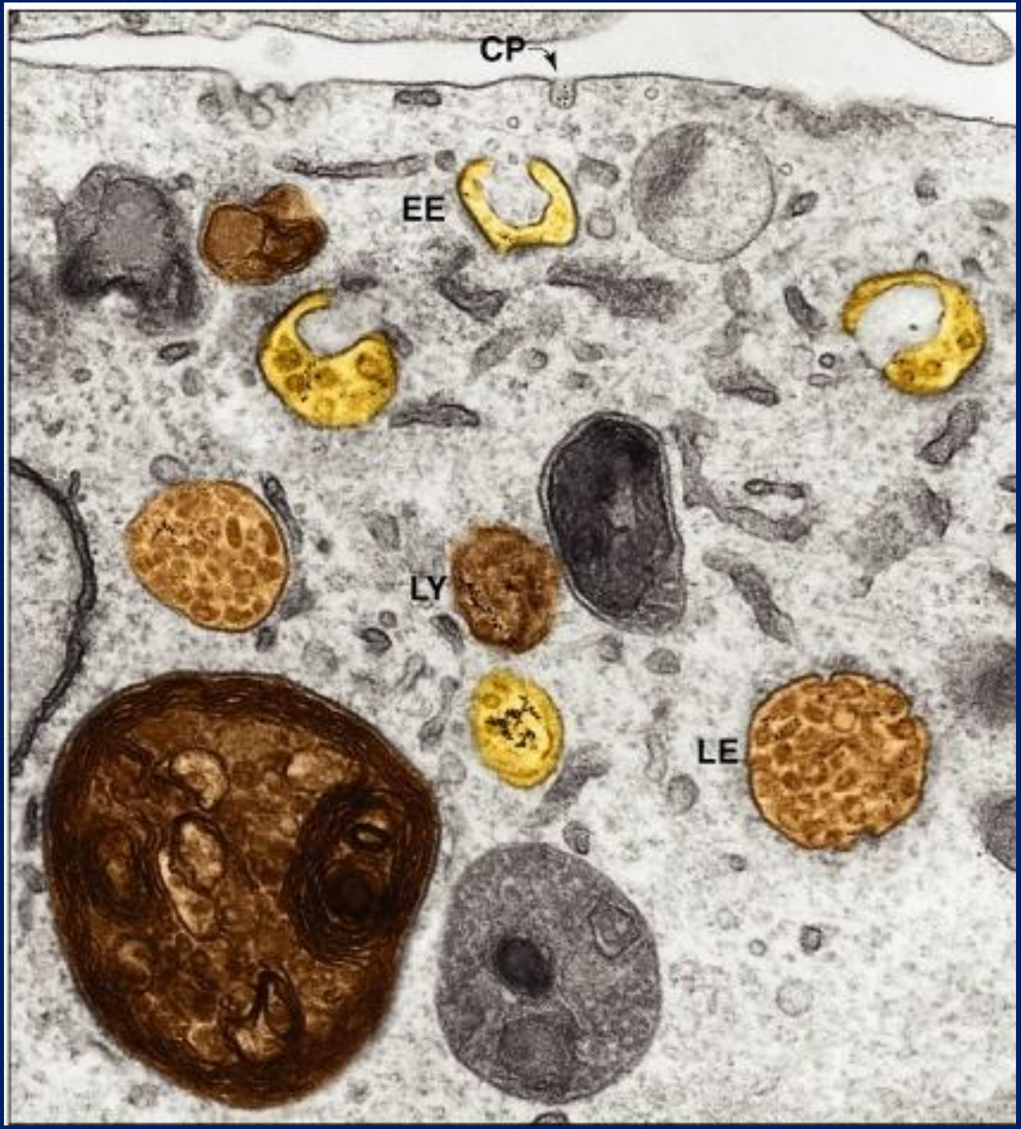
- ◆ Can be Non-clathrin/non-caveolae (Currently poorly understood)

Pinocytosis/ phagocytosis

- Pinocytosis: internalization of fluid
  - Actin dependent
  - Generated at sites of ruffling at the plasma membrane
  - Includes macropinocytosis (vesicles > 1 mm in diameter) and micropinocytosis (vesicles < 200nm in diameter)
- Phagocytosis: internalization of particles
  - Occurs in specialized cells ex. neutrophils and macrophages
  - Actin dependent
  - Clathrin independent
  - Particles > 0.5 mm in diameter

Pretty Colored Vesicles

- Gold dust through the endolytic pathway
  - coated pits (CP)
  - early endosome (EE)
  - late endosome (LE)
  - lysosome (LY)



Transport from the ER to the Golgi

When the protein is properly folded, COPII coated vesicles transport the proteins via the vesicular tubular cluster (vtc) to the cis-Golgi network.

- The COPII coating is removed (Sar1 hydrolyzes GTP) and the vesicles fuse with each other to form the vtc.
- The vtc is motored along microtubules that function like railroad tracks.
- The vtc fuses with the cis-Golgi network.

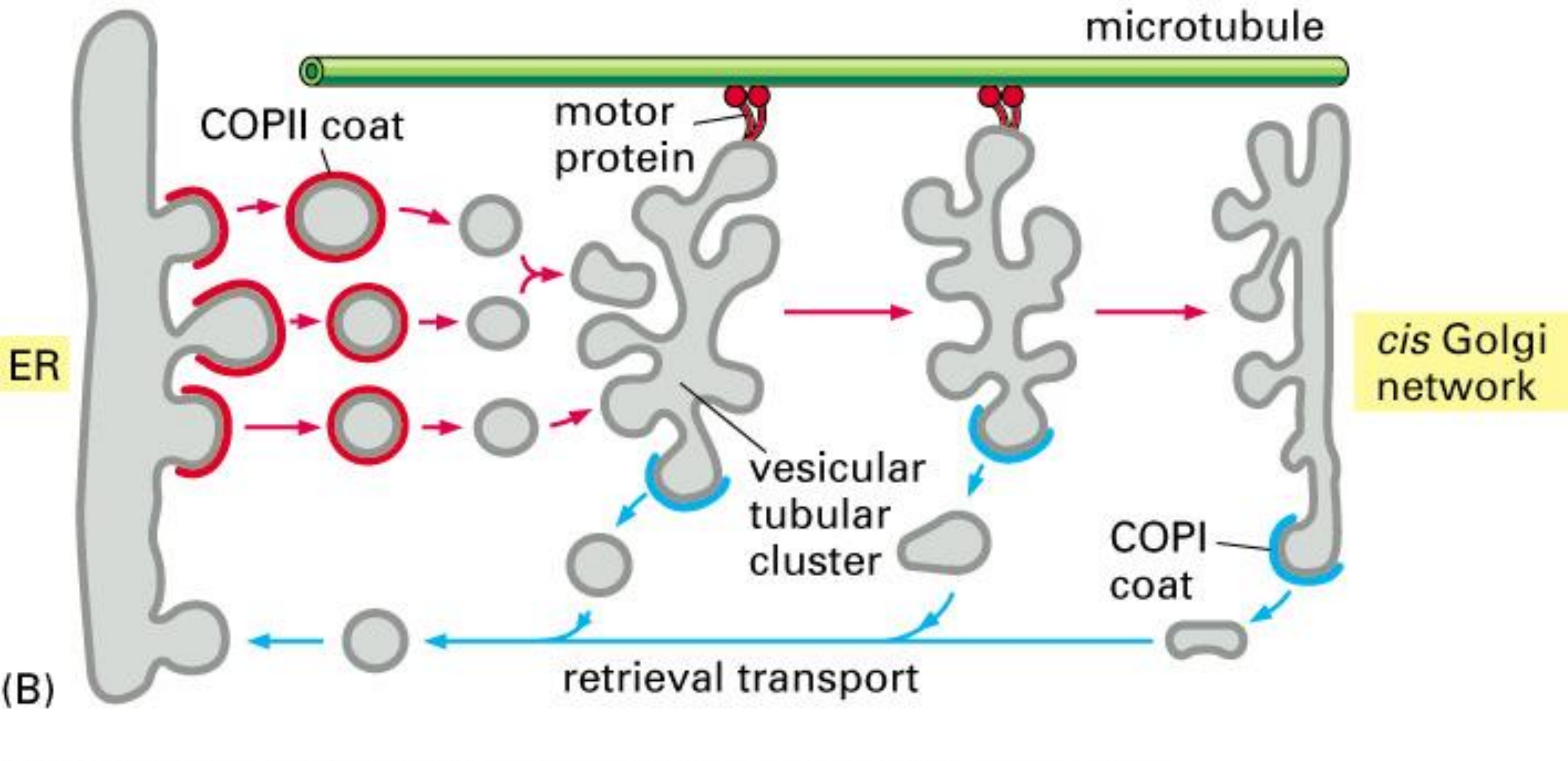
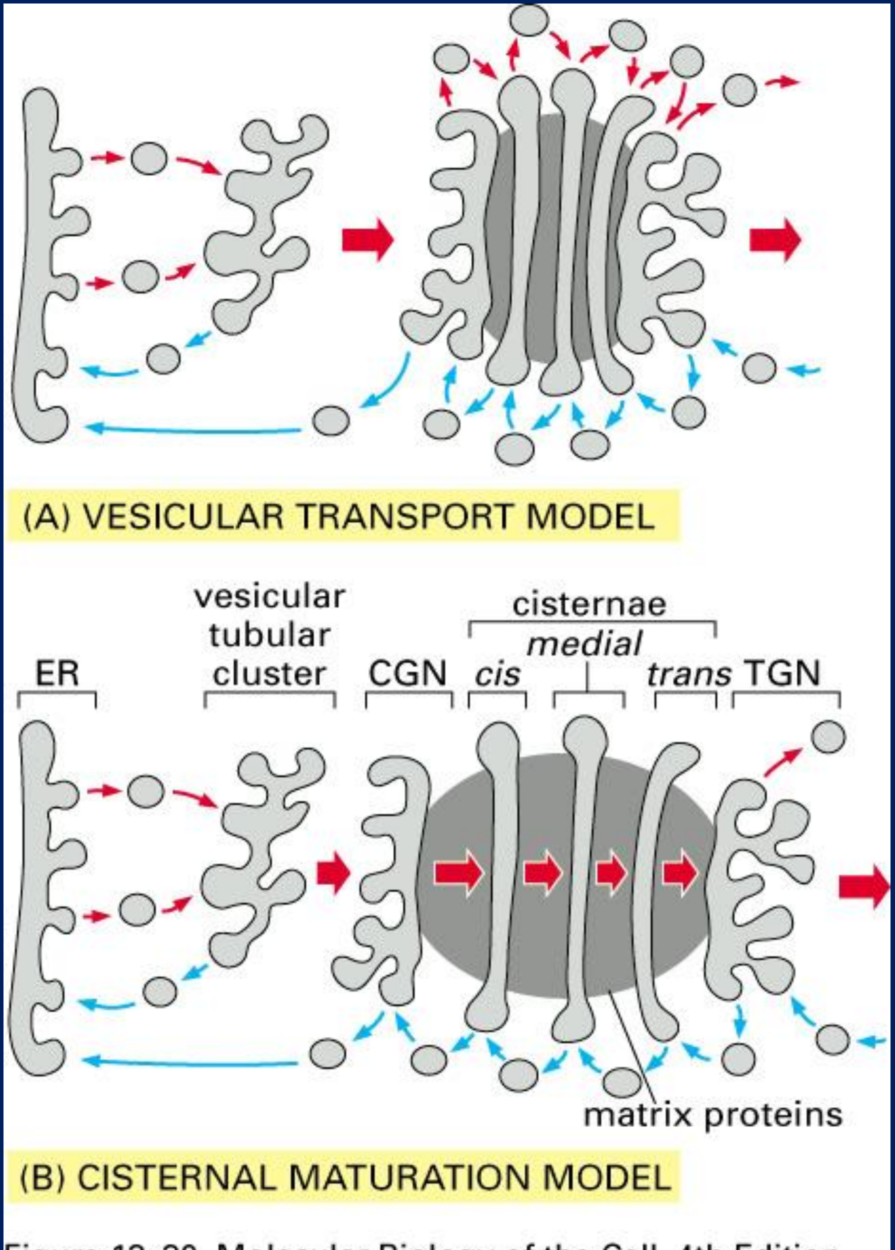


Figure 13–20 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Two Models For Cis to Trans-Golgi Progression

Traditional Model - Golgi is a static organelle. **Secretory proteins move forward in small vesicles**. Golgi resident proteins stay where they are.

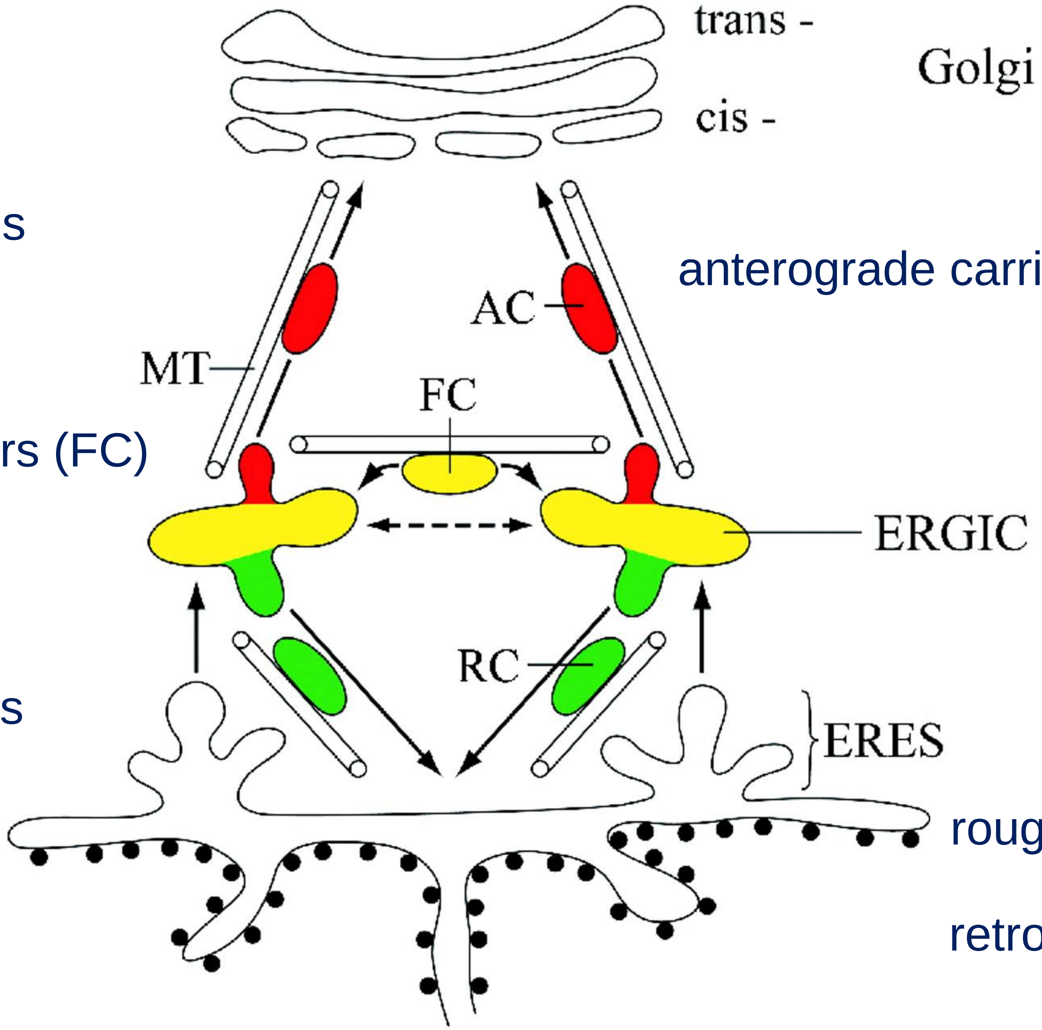


(A) VESICULAR TRANSPORT MODEL
ER
vesicular tubular cluster
CGN
cisternae
cis
medial
trans
TGN
matrix proteins
(B) CISTERNAL MATURATION MODEL

Figure 13–30. Molecular Biology of the Cell, 4th Edition.

"Radical" Model - Golgi is a dynamic structure. It only exists as a steady-state representation of transport intermediates. Secreted molecules move ahead with a cisterna. **Golgi resident proteins move backward to stay in the same relative position.**

Model of the organization of the early secretory pathway and sorting of anterograde and retrograde traffic in the ERGIC. The cycling protein GFP-ERGIC-53 (green) and anterograde cargo, such as VSV-G-GFP or ssDsRed (red), leave the ER at ER-exit sites (ERES)...



MT, microtubules

anterograde carriers (AC)

fast-moving carriers (FC)

ER-Golgi intermediate compartment (ERGIC)

ER-exit sites (ERES)

rough ER

retrograde carriers (RC)

Ben-Tekaya H et al. J Cell Sci 2005;118:357-367

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Journal of Cell Science
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Early Secretory Pathway - Forward and Retrograde Traffic

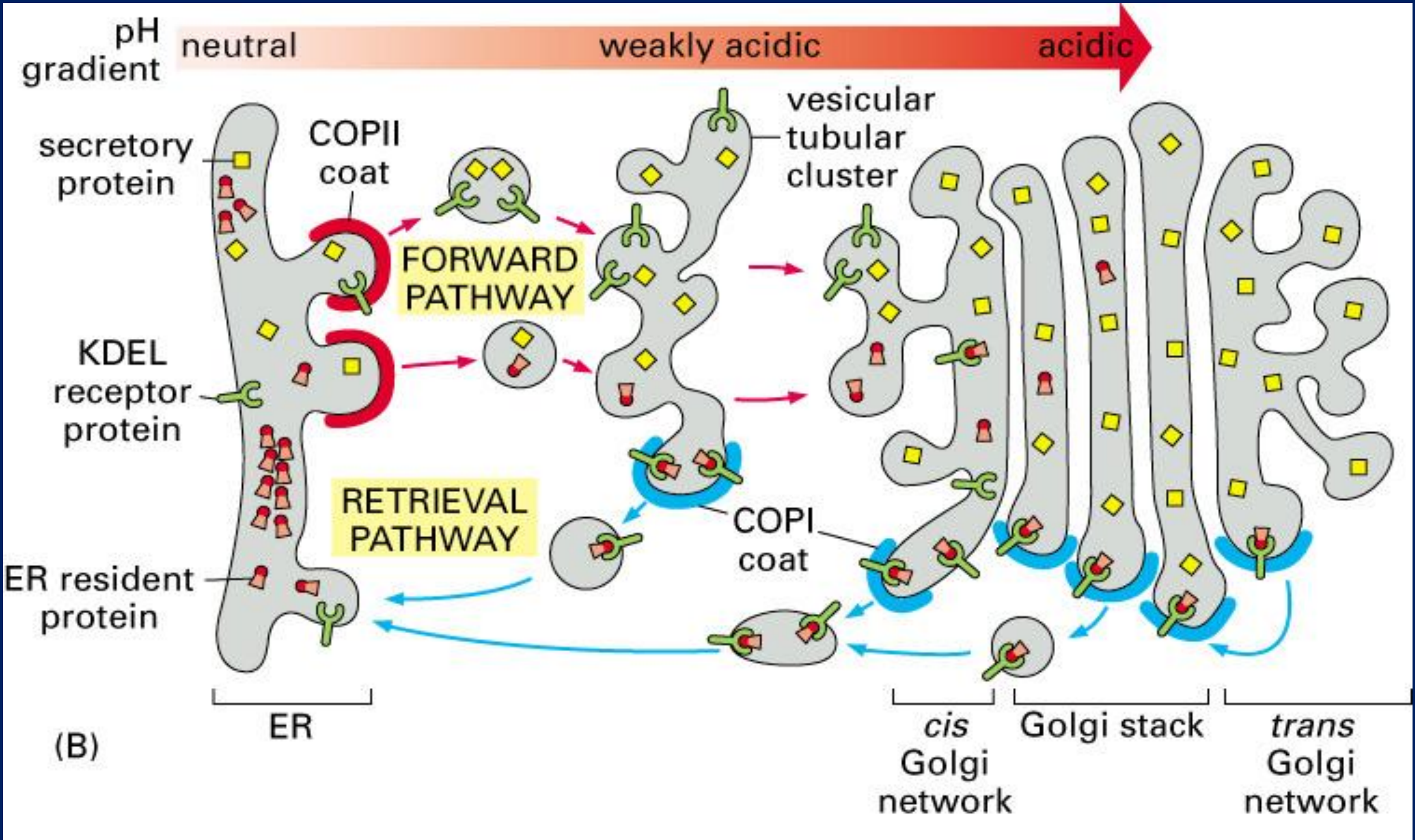
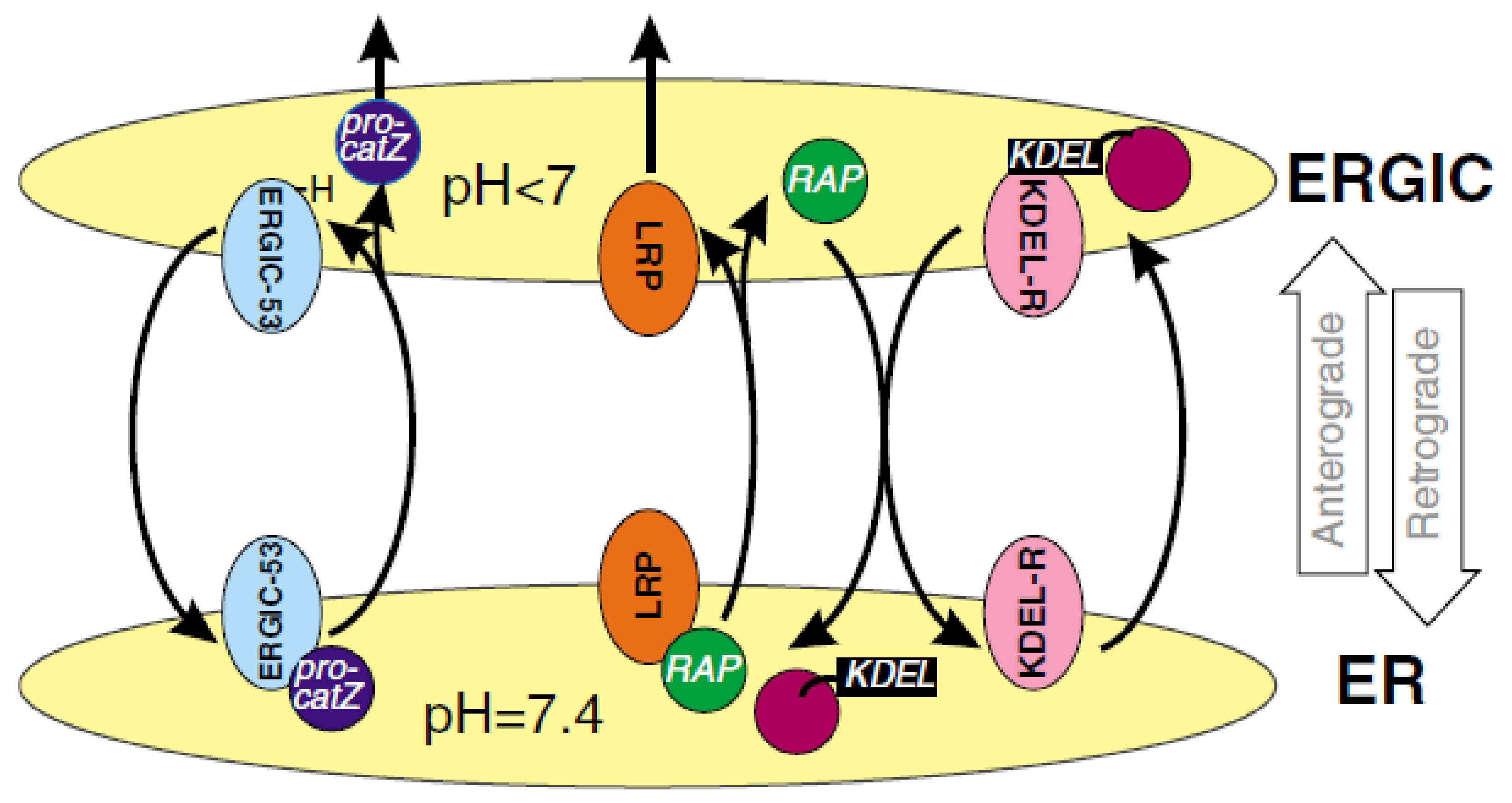


Figure 13–21 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

pH-dependent sorting between the ER and ERGIC.



KDEL-receptors bind to KDEL-bearing proteins in the low pH environment of the Golgi and release that Cargo in the neutral pH of the ER.

pH probably alters KDEL receptor conformation - regulating cargo binding and inclusion in COPI vesicles.

ER→Golgi transport

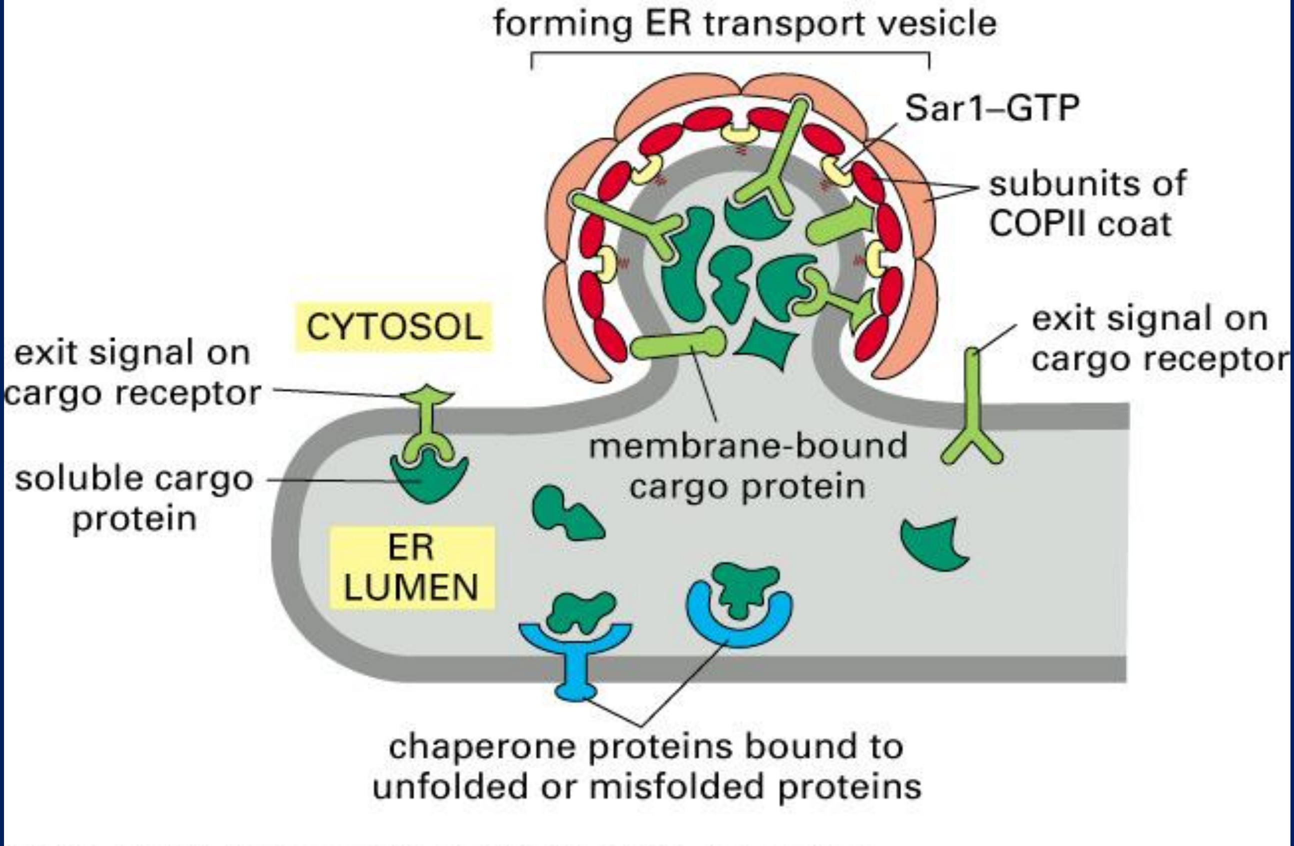

forming ER transport vesicle
Sar1–GTP
subunits of COPII coat
exit signal on cargo receptor
CYTOSOL
exit signal on cargo receptor
soluble cargo protein
membrane-bound cargo protein
ER LUMEN
chaperone proteins bound to unfolded or misfolded proteins

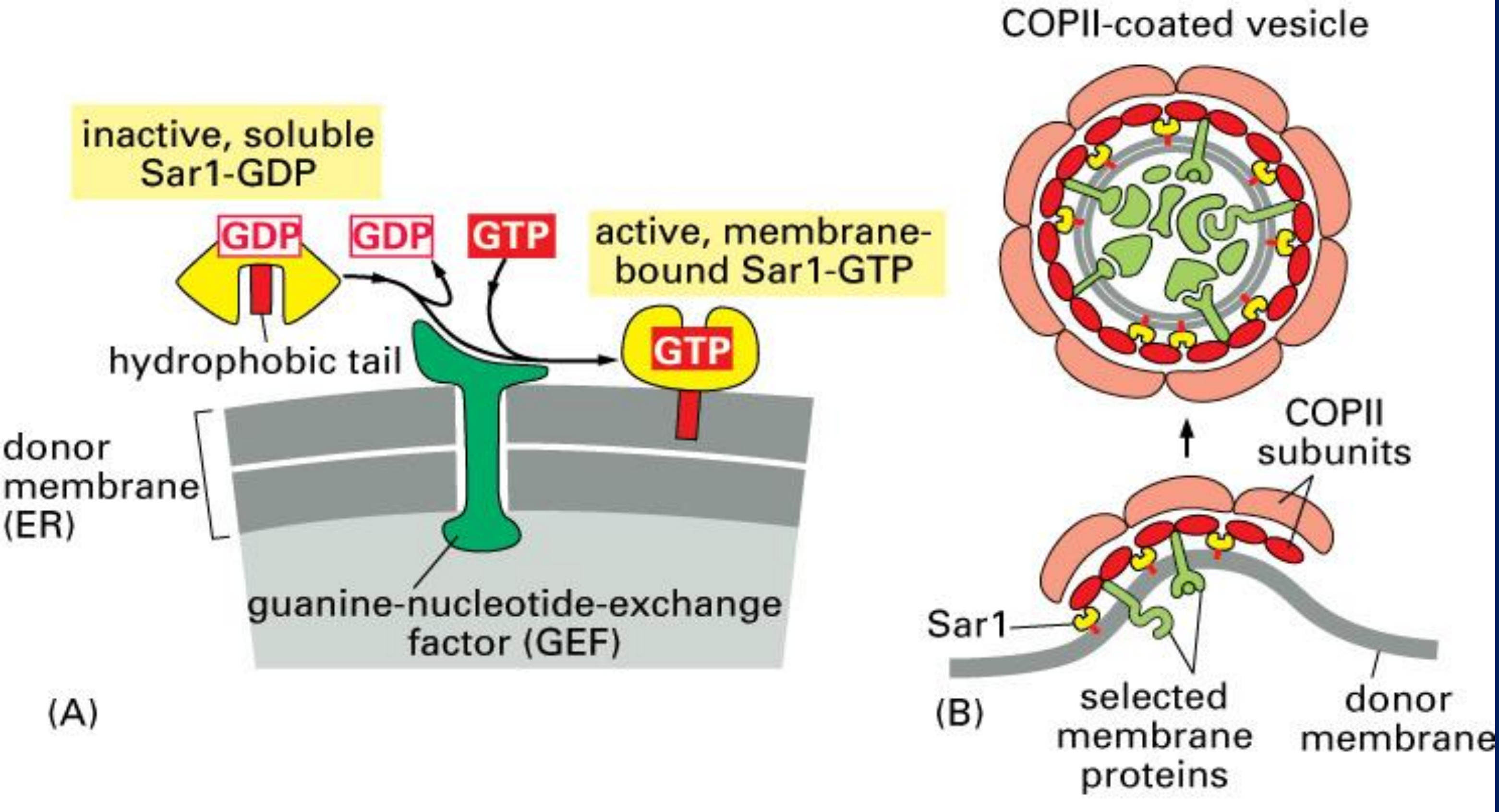
Figure 13–17. Molecular Biology of the Cell, 4th Edition.

Membrane and cargo molecules become concentrated in transport vesicles as they leave the ER.

Membrane proteins interact with components of the COPII coat through exit signals on their cytosolic tails.

Soluble proteins bind to certain concentrated membrane proteins known as cargo receptors.

Small GTPases Regulate Budding


COPII-coated vesicle
inactive, soluble Sar1-GDP
GDP
GDP
GTP
active, membrane-bound Sar1-GTP
GTP
hydrophobic tail
donor membrane (ER)
guanine-nucleotide-exchange factor (GEF)
(A)
COPII subunits
Sar1
(B)
selected membrane proteins
donor membrane

Sar1 is a small GTPase that acts to recruit coat proteins.

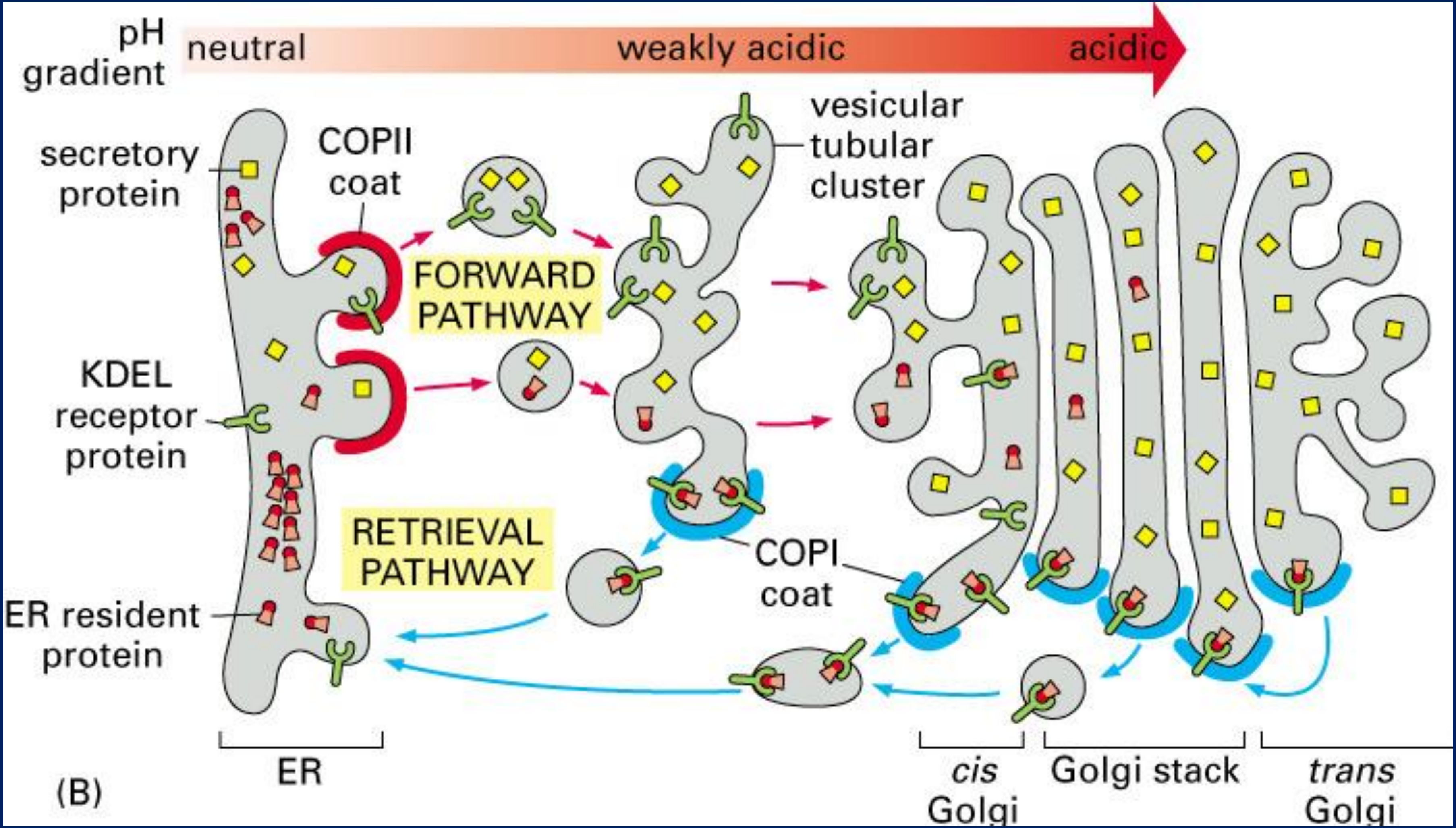
Sar1-GDP is converted to Sar1-GTP by a transmembrane GEF.

GTP binding triggers insertion of fatty acid tail of Sar1 into membrane.

Sar1-GTP on the membrane recruits COPII proteins, resulting in membrane curvature.

Pinching-off occurs to release the free vesicle. Uncoating occurs.

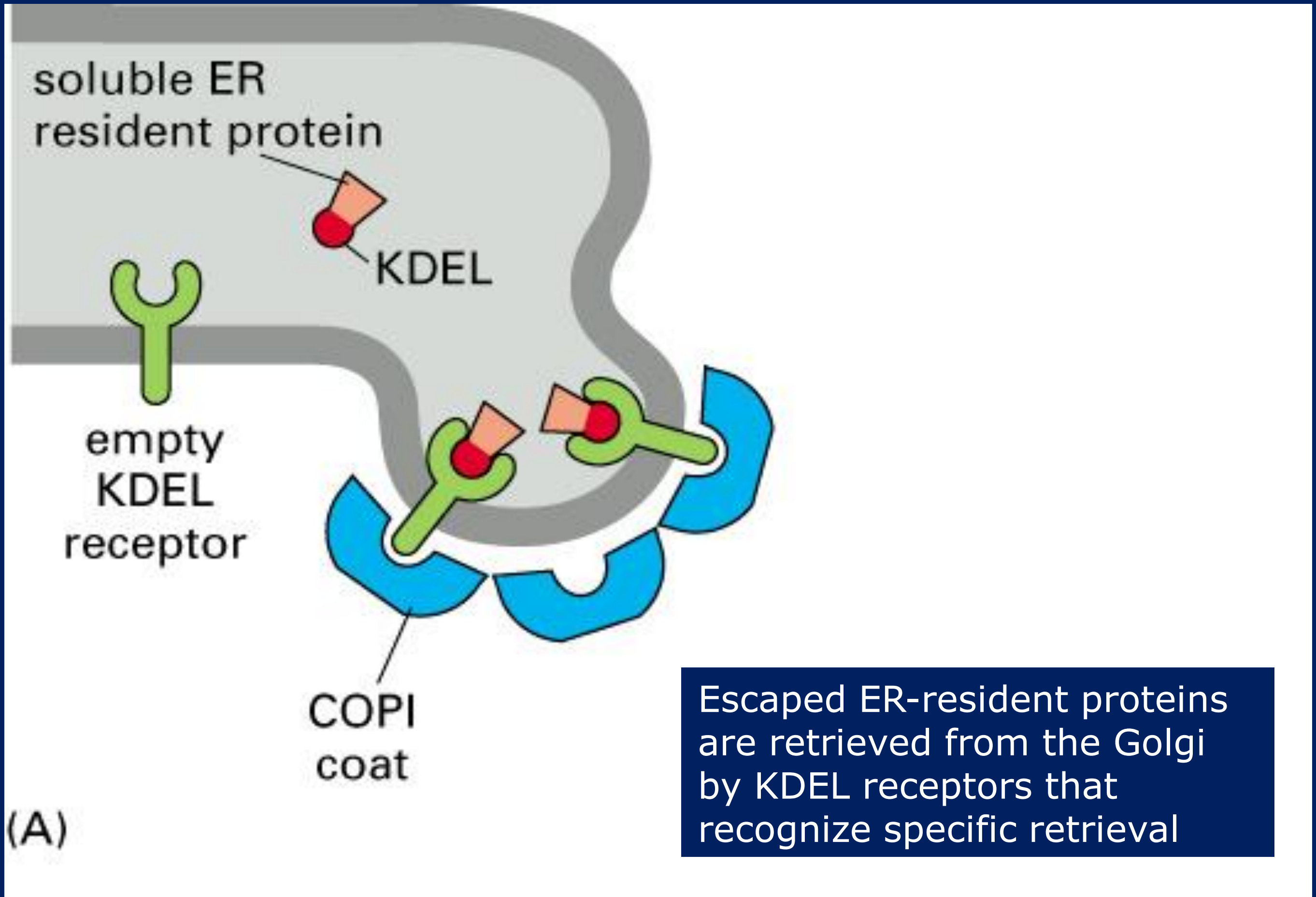
Some proteins exiting the ER **are returned** to the ER by COPI coated vesicles.



Example : ER chaperones like BiP that are mistakenly transported.

This example describes the situation of BiP. BiP has **the signal sequence, KDEL**. When BiP escapes the ER, it associates with the KDEL receptor. The slightly acid environment of the vtc and Golgi favor this association. When the returning vesicle fuses with the ER, the neutral pH of the ER causes BiP to dissociate from the receptor.

Retrieval of ER Resident Proteins is Receptor-Mediated

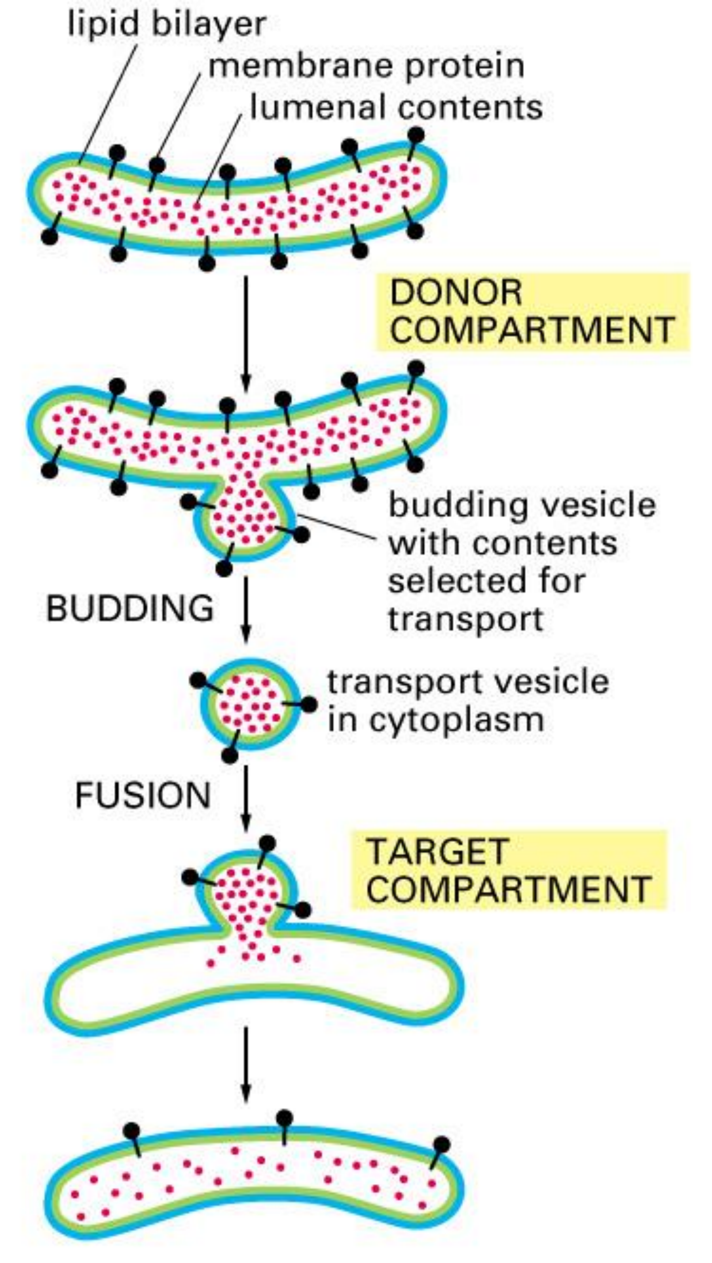


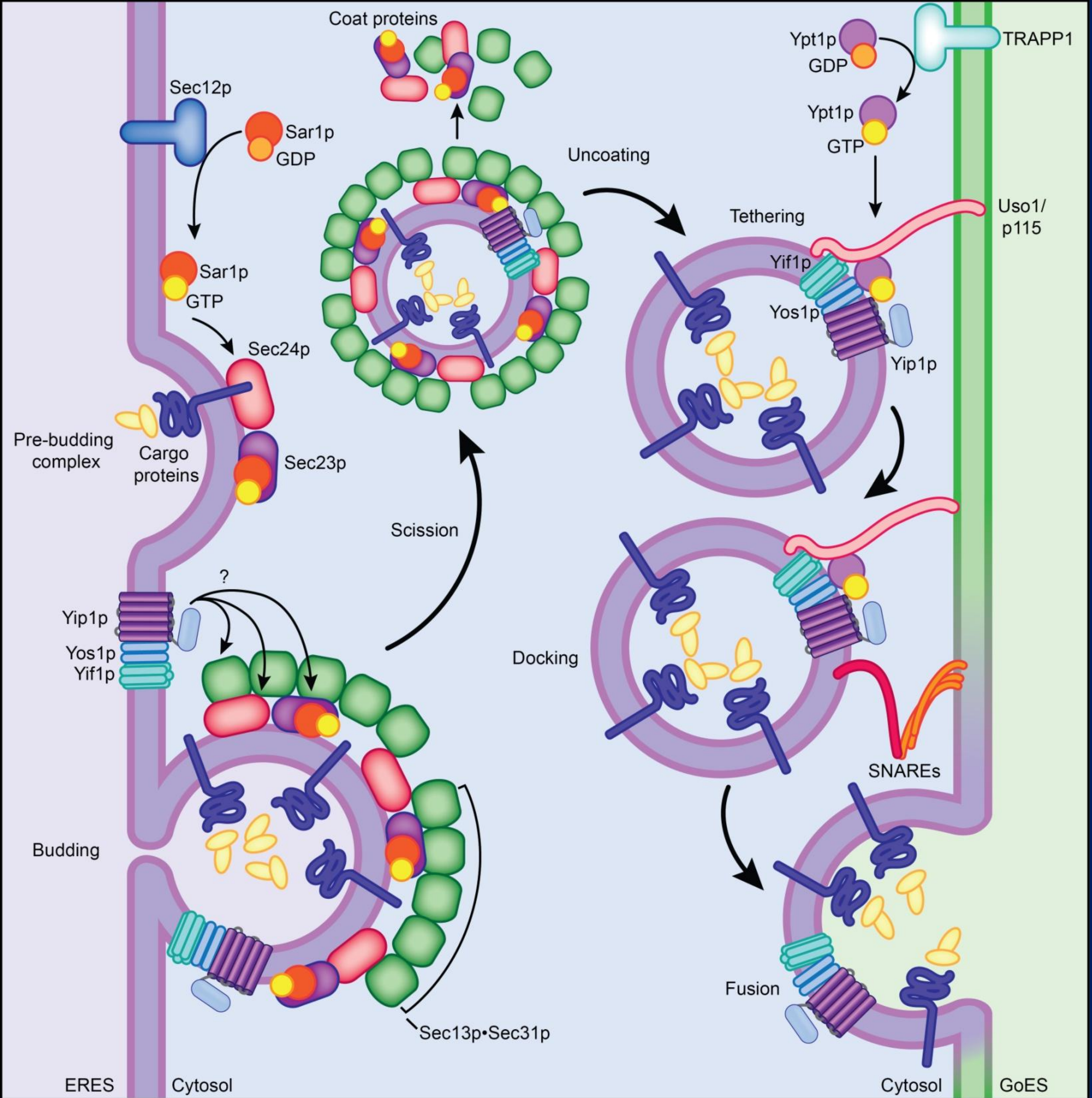
Escaped ER-resident proteins are retrieved from the Golgi by KDEL receptors that recognize specific retrieval

Figure 13–21 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

Обеспечение направленности переноса везикул

Vesicular Transport Proceeds by Budding and Fusion

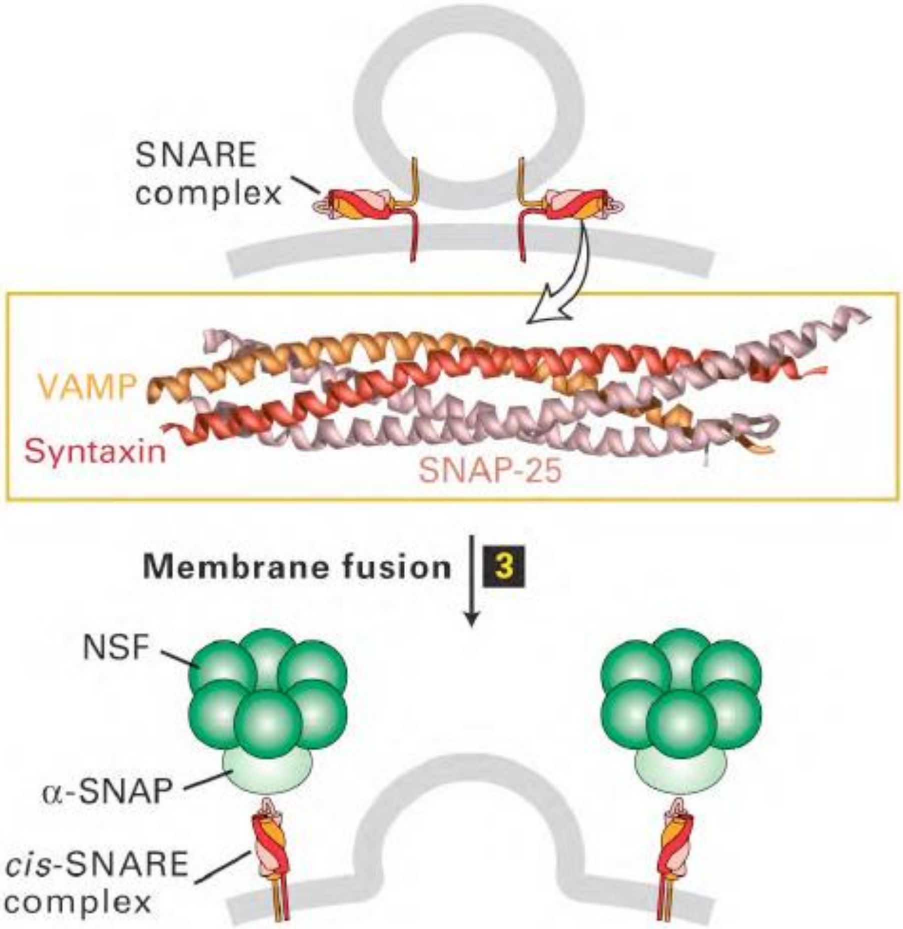




ER exit sites (ERES)

Goldgi exit sites (GoRES)

Assembly of SNARE complexes is required for membrane fusion



evidence for involvement of SNARE proteins in membrane fusion:

-neurotoxins (BoNT, TeTX) that specifically cleave SNARE proteins

-yeast or mouse mutants

-antibodies etc. against SNAREs

-SNARE-containing proteoliposomes

SNARE= Soluble NSF Attachment Protein (SNAP) Receptors

v-SNARE: vesicle SNARE
t-SNARE: target membrane SNARE

Step 2: Fusion of lipid bilayers; cis-SNARE complexes remain

NSF Unfolds SNARE Complexes, Allowing Them To Recycle

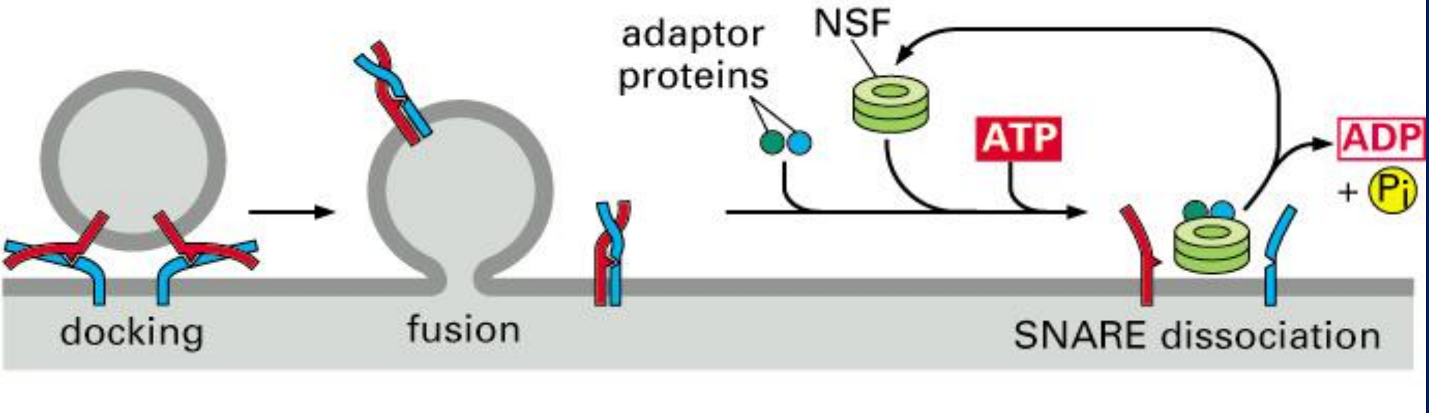


Figure 13–13. Molecular Biology of the Cell, 4th Edition.

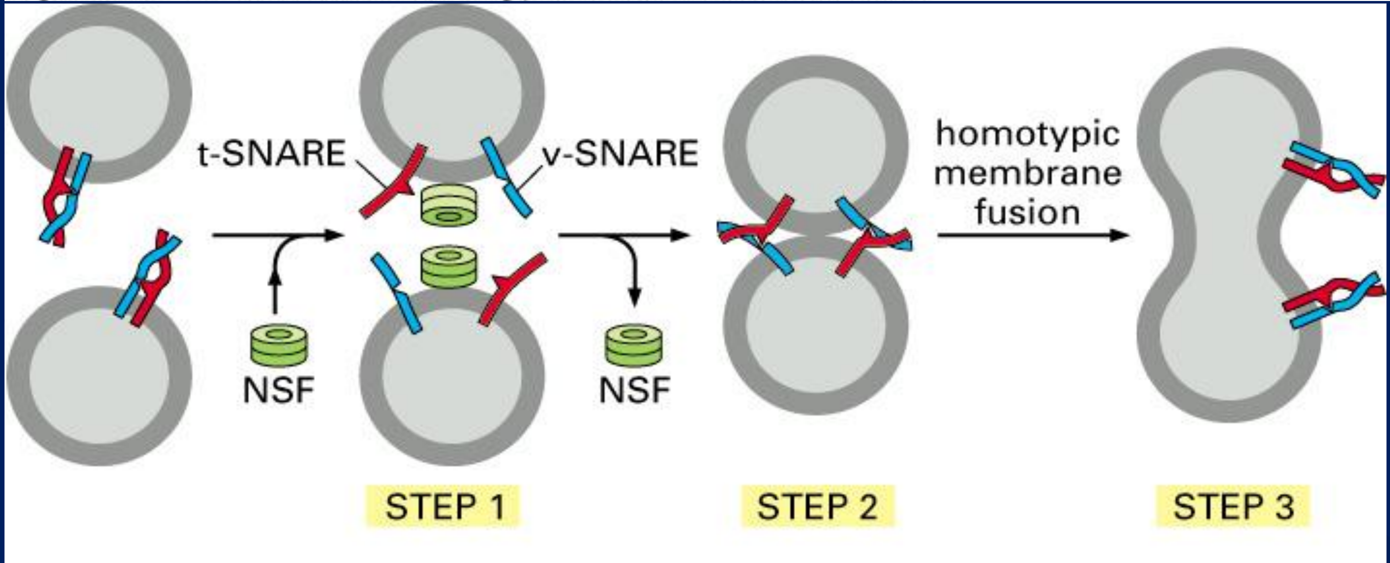


Figure 13–19. Molecular Biology of the Cell, 4th Edition.

NSF, a AAA-ATPase, associates with the snare complex via adaptor proteins and dissociates the complex in an ATP dependent manner.

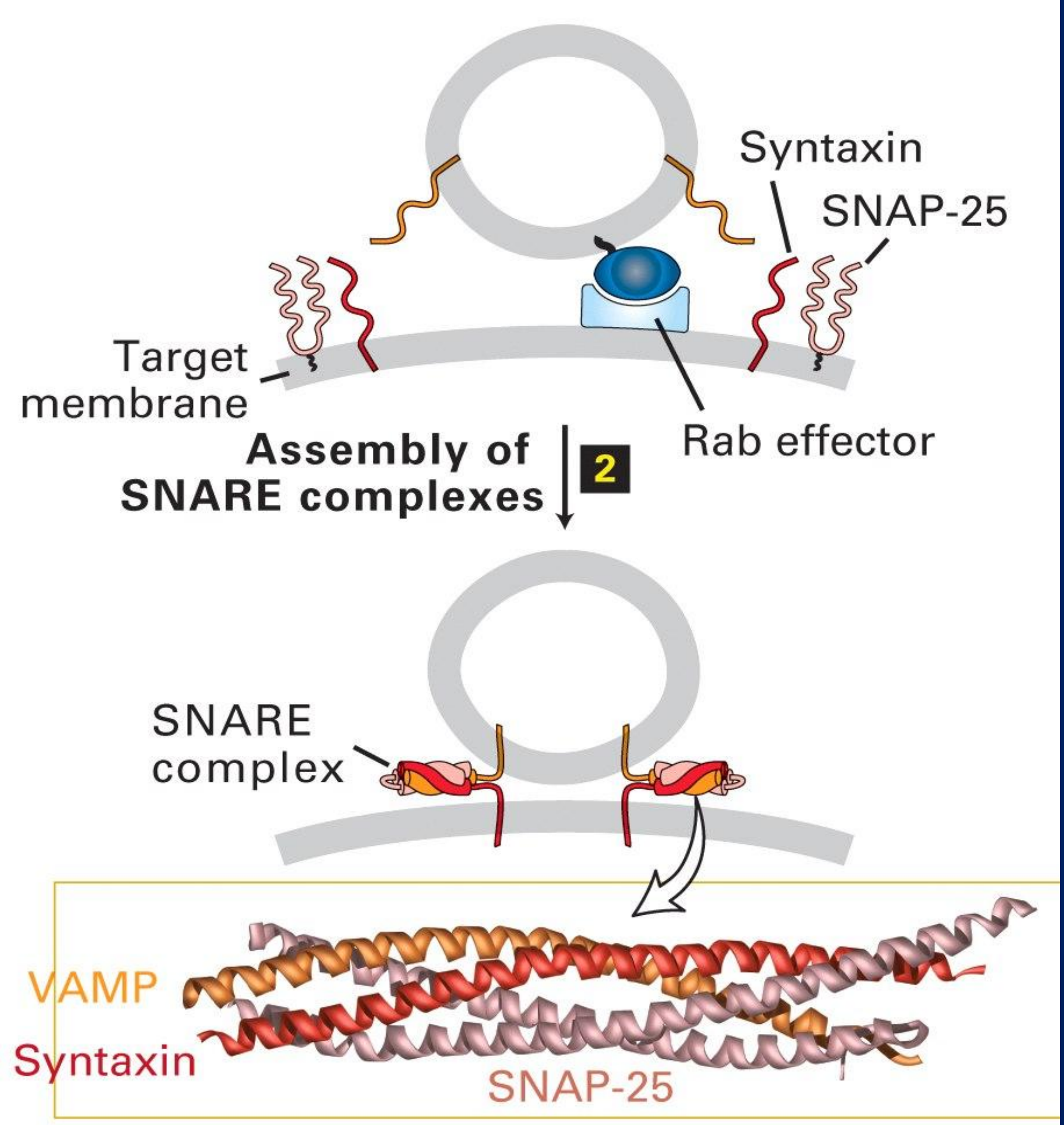
Without this reaction snares can be used only once!

Specific fusion of intracellular vesicles involves a conserved set of fusion proteins

SNARE proteins mediate **both specificity of fusion and the fusion process itself.**

V-SNARE (vesicle), T-SNARE (target), and SNAP25 form a 4-helix bundle (coiled-coil).

Specific Rab proteins are required for specific fusion events. Their exact role is unknown, but they probably function to recruit other effectors that participate in docking and fusion. **Rab-GTP is the active form.**



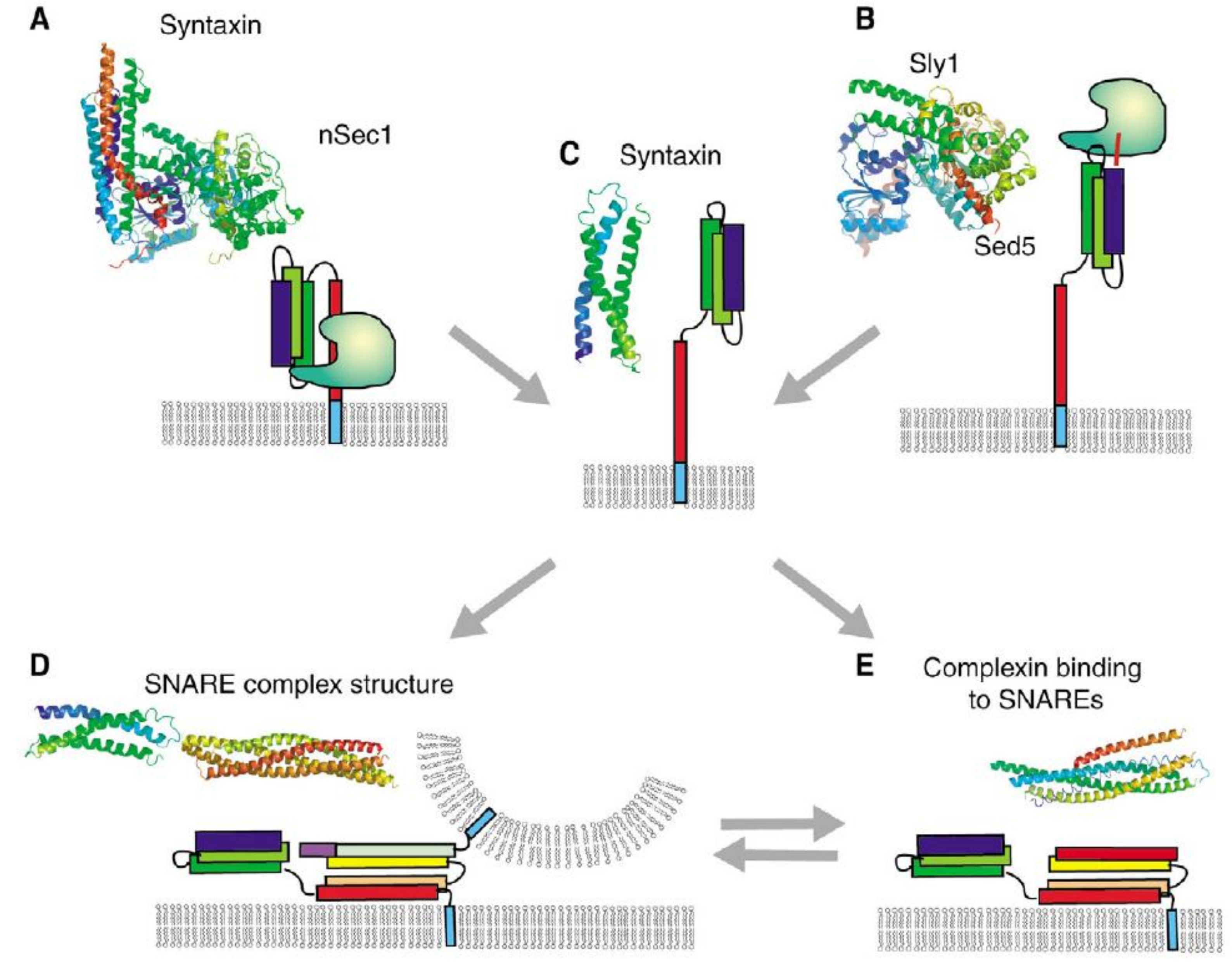


Fig. 2. SNARE complex assembly and its control. (A) Interaction of syntaxin with nSec1, also known as Munc18 (1DN1) (Yang et al., 2000). nSec1 binds to the closed conformation of syntaxin, regulating its availability. (B) Binding of Sly1 to the N-terminal peptide of the SNARE Sed5 may function similarly (1MQS) (Bracher and Weissenhorn, 2002). (C) The open conformation of the syntaxin molecule (as shown in Fig. 1C). (D) The assembled SNARE complex modeled between two membranes (1SFC) (Sutton et al., 1998). (E) Interaction of complexin with the SNARE complex might regulate exocytosis (1KIL) (Chen et al., 2002).

Intracellular cholesterol transport

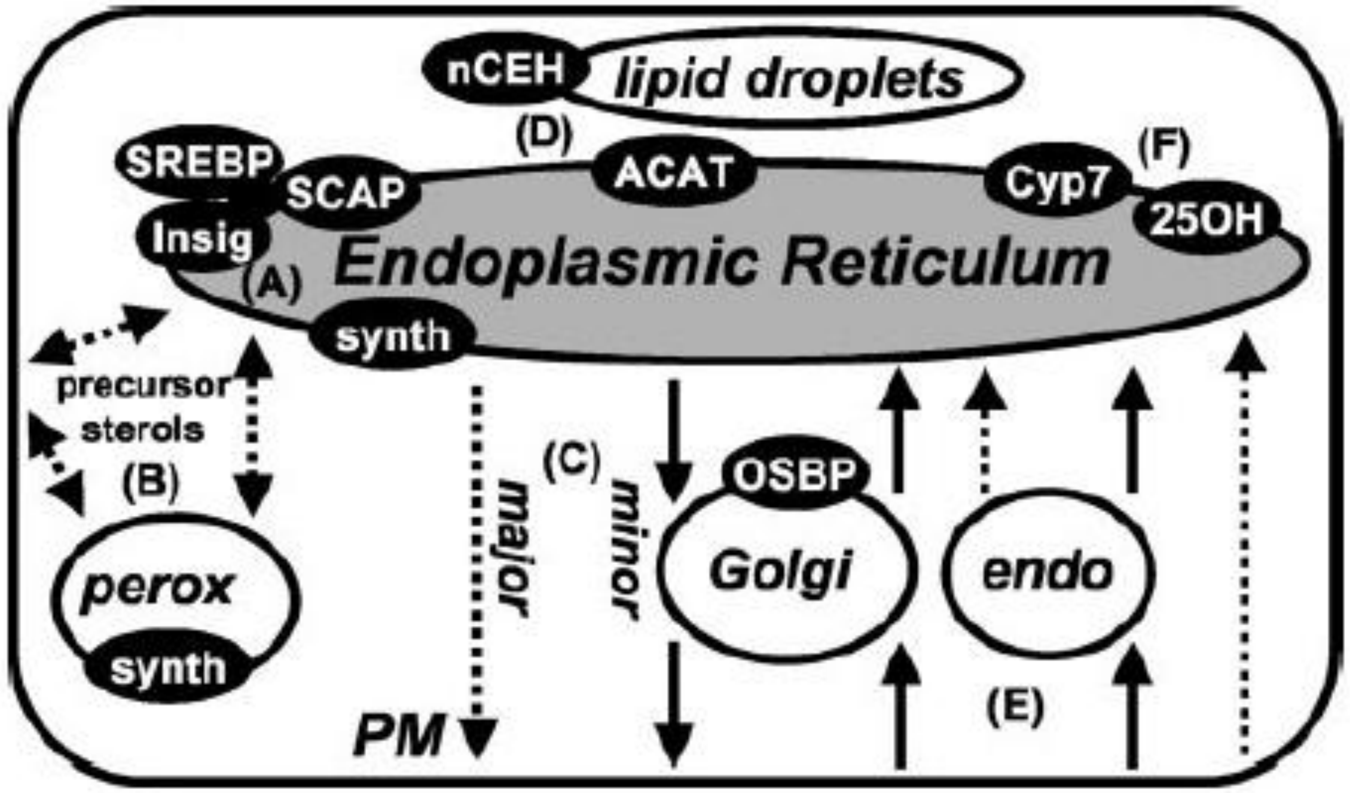
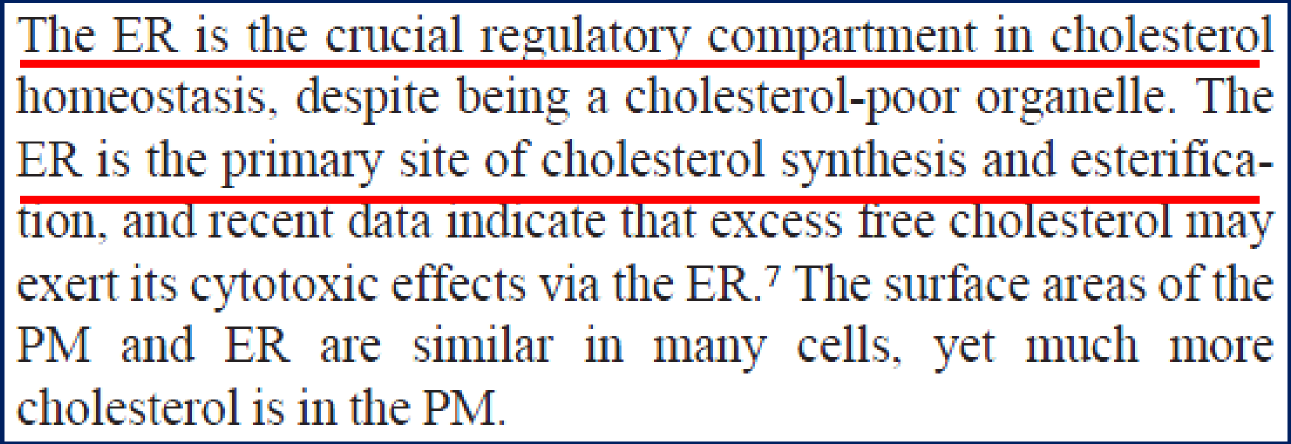
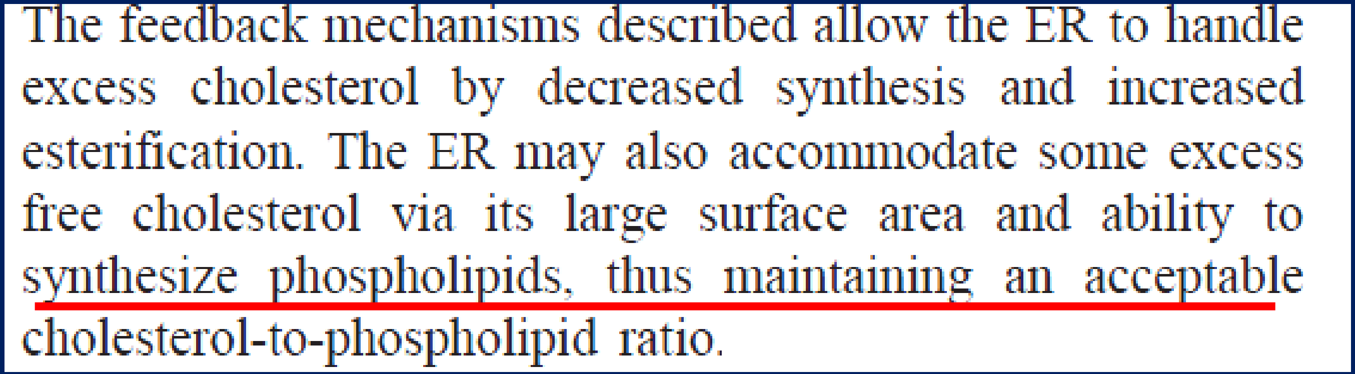


Figure 1. Endoplasmic reticulum (ER) cholesterol transport.



The ER is the crucial regulatory compartment in cholesterol homeostasis, despite being a cholesterol-poor organelle. The ER is the primary site of cholesterol synthesis and esterification, and recent data indicate that excess free cholesterol may exert its cytotoxic effects via the ER.[7] The surface areas of the PM and ER are similar in many cells, yet much more cholesterol is in the PM.



The feedback mechanisms described allow the ER to handle excess cholesterol by decreased synthesis and increased esterification. The ER may also accommodate some excess free cholesterol via its large surface area and ability to synthesize phospholipids, thus maintaining an acceptable cholesterol-to-phospholipid ratio.

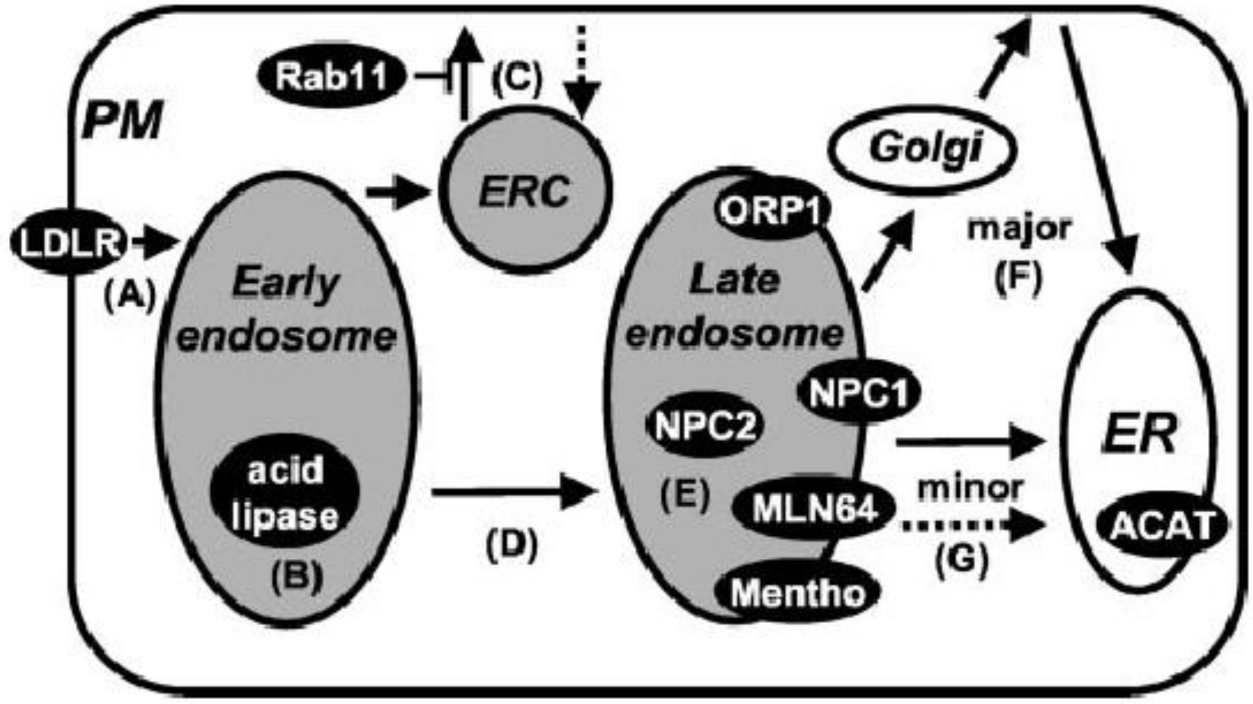
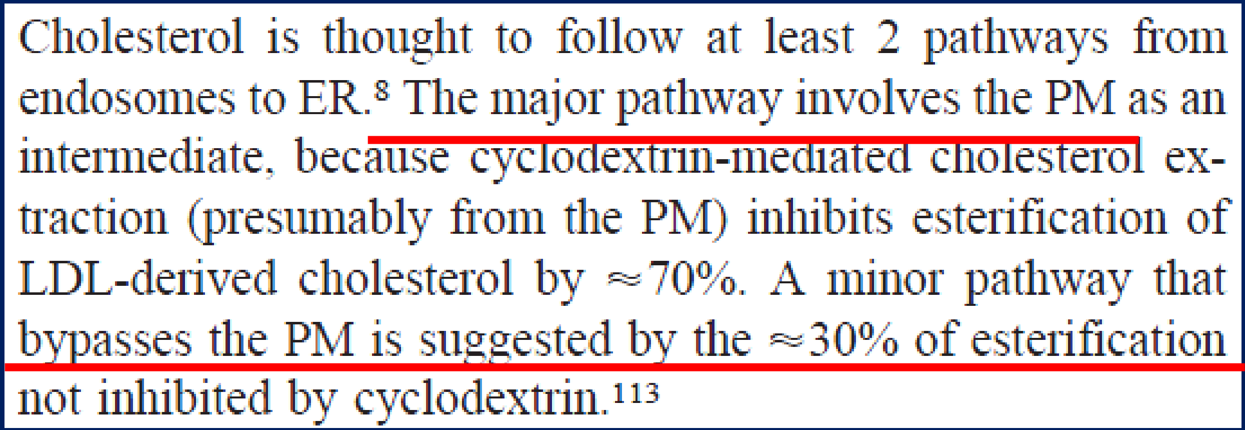


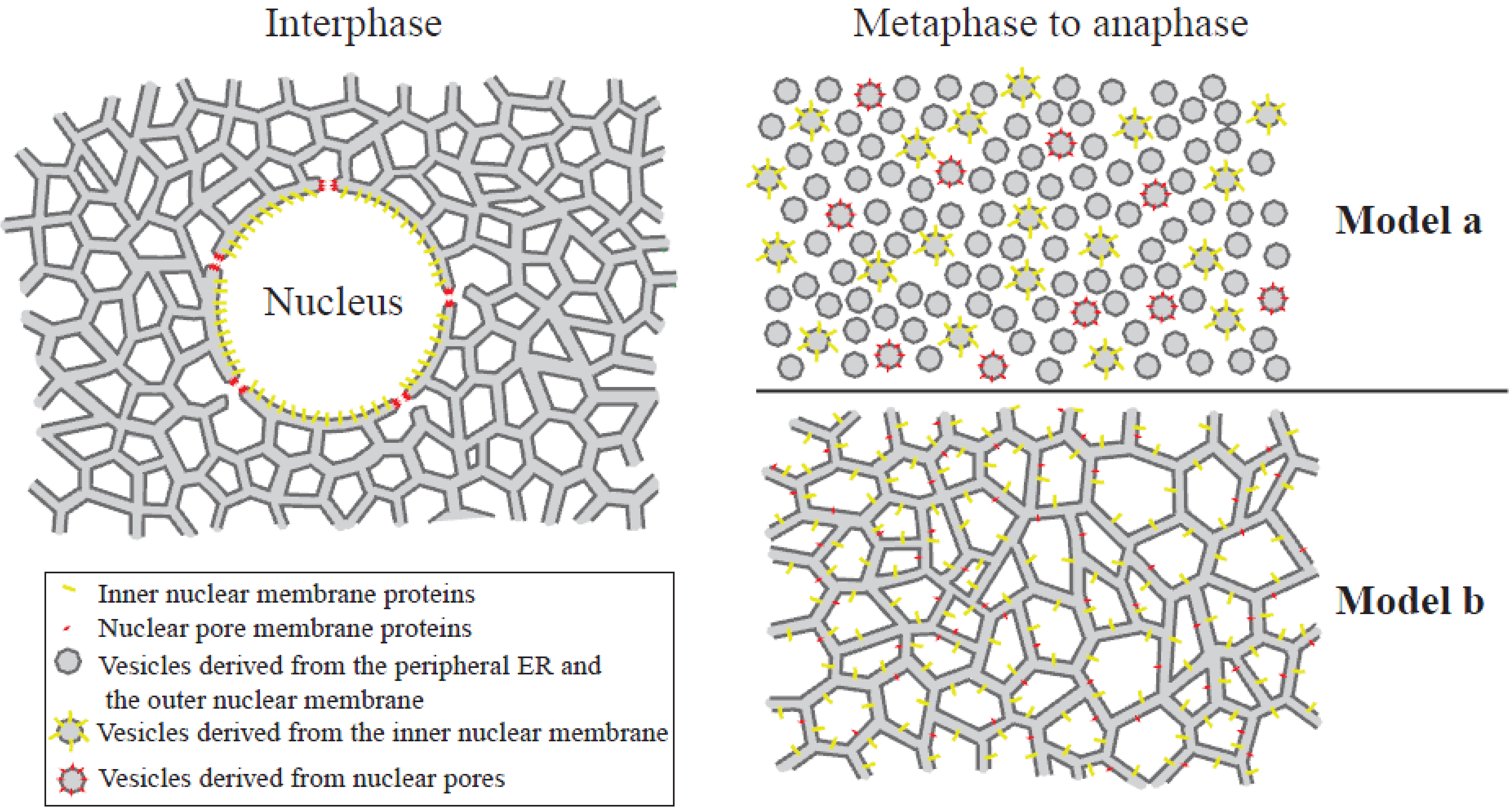
Figure 2. Endosomal cholesterol transport.



Cholesterol is thought to follow at least 2 pathways from endosomes to ER.[8] The major pathway involves the PM as an intermediate, because cyclodextrin-mediated cholesterol extraction (presumably from the PM) inhibits esterification of LDL-derived cholesterol by ≈70%. A minor pathway that bypasses the PM is suggested by the ≈30% of esterification not inhibited by cyclodextrin.[113]

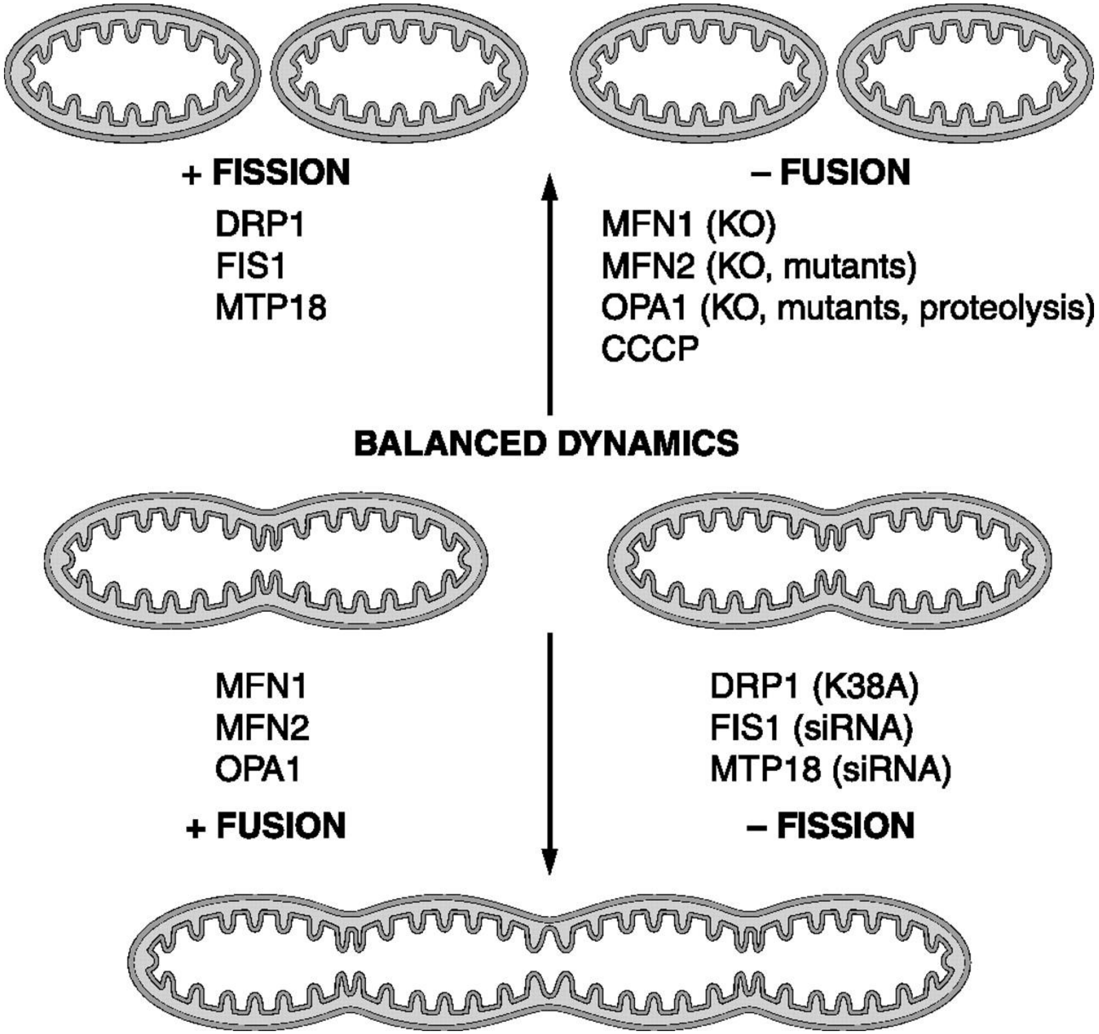
Динамика органелл

Models of ER segregation in proliferating animal cells.



- Inner nuclear membrane proteins
- Nuclear pore membrane proteins
- Vesicles derived from the peripheral ER and the outer nuclear membrane
- Vesicles derived from the inner nuclear membrane
- Vesicles derived from nuclear pores

Mitochondrial morphology is partly dependent on a proper balance between fusion and fission processes.



+ FISSION

DRP1
FIS1
MTP18

– FUSION

MFN1 (KO)
MFN2 (KO, mutants)
OPA1 (KO, mutants, proteolysis)
CCCP

BALANCED DYNAMICS

MFN1
MFN2
OPA1

+ FUSION

DRP1 (K38A)
FIS1 (siRNA)
MTP18 (siRNA)

– FISSION

Liesa M et al. Physiol Rev 2009;89:799-845

Physiological Reviews

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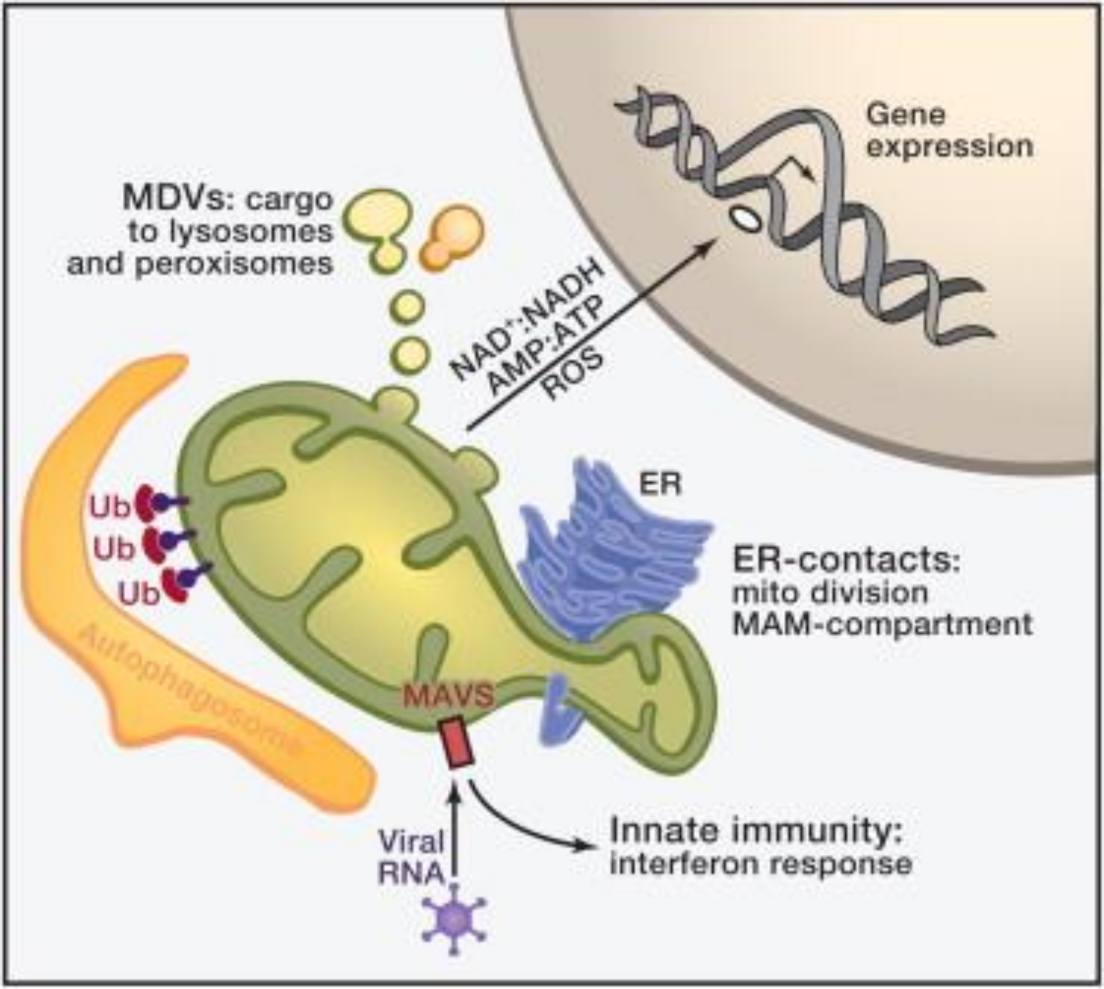
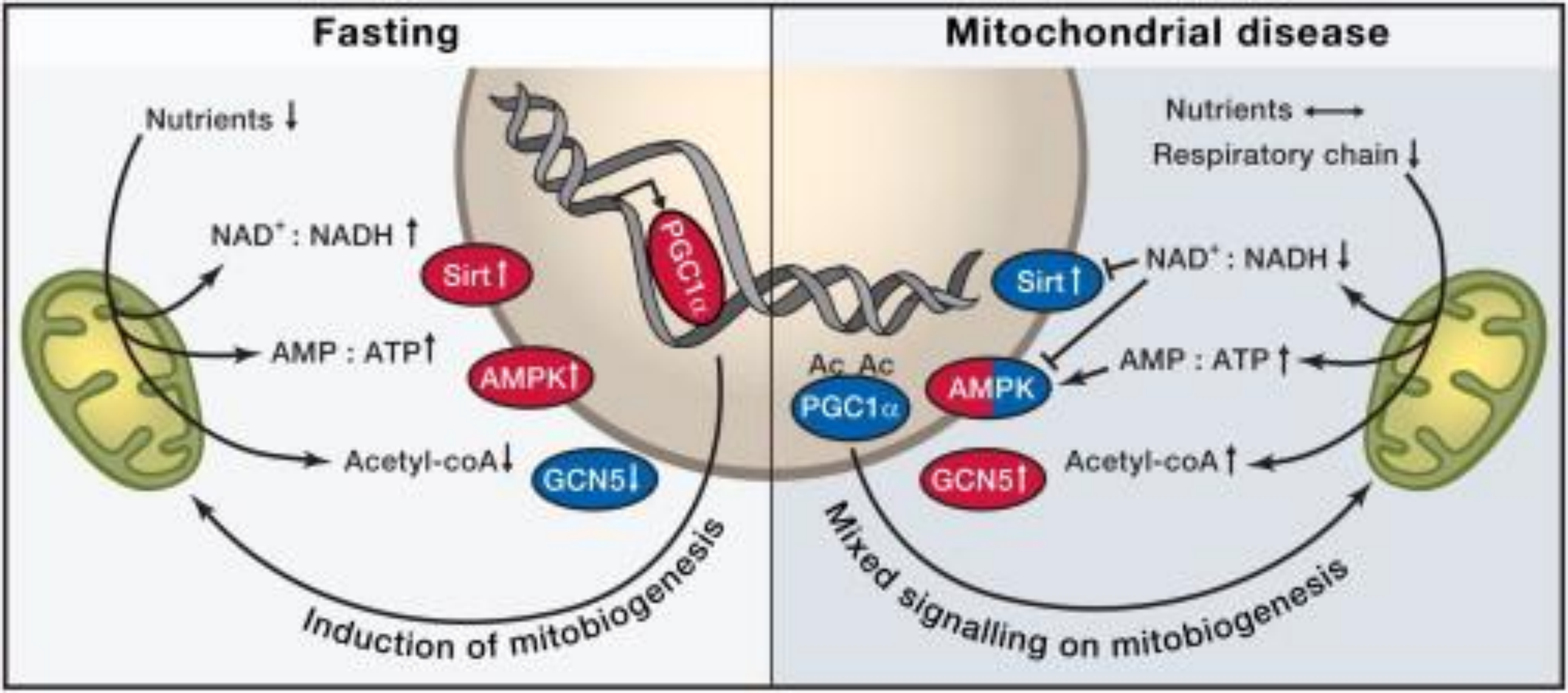
Interorganellar Communication



Nutrient Sensors in Fasting and Their Roles in Mitochondrial Disease



Взаимодействие компонентов цитоскелета для транспорта митохондрий в аксонах